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SEVENTH FRAMEWORK PROGRAMME

Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

Deliverable D4.2-3: Report/manuscript on benthic macroflora indicators for coastal waters, including classification boundaries, definition of reference conditions and uncertainty

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Content

Non-technical summary

The requirement of the EU Water Framework Directive (WFD) to classify all surface water bodies according to their "ecological status" has shifted management objectives from merely pollution control to ensuring ecosystem integrity as a whole. This requires that complex and dynamic biological communities are quantified into a single numeric score, which measures the status of the system relative to established reference conditions. This is being carried out within a large number of water body types. Because each Member State can develop methods for water body quality assessment fulfilling the complex requirements of the WFD, a wide variety of methods throughout Europe differing greatly in the way of defining reference conditions, type vs. site-specific assessment, the number and nature of indices (metrics) used, etc. have mushroomed. Several indices using macrophytes to assess the ecological status of coastal waters were developed prior the start of WISER project, but some have been developed during the project. It is crutial to evaluate the robustness and reliability of the different indices developed. This is to be done mainly through quantification of pressure-indicator responses (see Deliverable 4.2-2) and uncertainty analysis, a powerful tool that allows the identification of the factors contributing to the potential misclassification of the ecological status class of water bodies.

The objectives of this deliverable are: (1) to summarize the characteristics of the macroflora classification methods studied in WISER; (2) to describe the new macroflora classification methods developed within WISER Project; and (3) to determine which sources of variability (factors) associated with the sampling design different coastal WFD monitoring programmes using classification methods based on macrophytes, most greatly influence the classifications of water bodies.

WISER consortium is using 7 different classification methods based on macrophyte metrics: i) "Multi Species Maximum Depth Index" (MSMDI, North Atlantic - Norway), ii) "RSLA" (North Atlantic-Norway), iii) "Eelgrass Depth Limit" (EDL, Baltic Sea - Denmark), iv) "*Posidonia oceanica* Multivariate Index" (POMI, Mediterranean - Spain and Croatia), v) "Ecological Index" (EEI-c, Black Sea – Bulgaria; Mediterranean Sea- Greece, Cyprus, Slovenia), vi) "Marine Macroalgae Assessment Tool" (MarMAT, North Atlantic- Portugal) and vii) "Rocky Intertidal Community Quality Index" (RICQI, North Atlantic - Spain). MarMAT, RICQI and RSLA have been developed in collaboration with WISER Project, and the rest have been developed prior the onset of WISER Project. The analysis of the uncertainty associated to the ecological quality status classification of water bodies is a good proxy to identify and quantify the factors that may affect the risk of misclassification. When applied to macrophyte monitoring programs, we have observed that the main sources of uncertainty are mostly associated to the sampling spatial scales, while temporal or human-induced errors seem to be less relevant. As a guide for taking management decisions, adequate sampling designs that include replication at different spatial scales within water bodies may substantially reduce this uncertainty. In some cases, it is not increasing the sampling effort but distributing it more efficiently within the allocated time and budget constrains that we will be able to maximize the confidence of estimations when assessing ecosystem health under the WFD.

1. Introduction

The requirement of the EU Water Framework Directive (WFD; Directive 2000) to classify all surface water bodies according to their “ecological status” has precipitated a fundamental change in management objectives from merely pollution control to ensuring ecosystem integrity as a whole (Hering et al. 2010). The concept of “ecological status”, as defined by the WFD, is the quality of the structure and functioning of aquatic ecosystems associated with surface waters (Bennett et al. 2011). Rather than focus only on limited aspects of chemical quality, the WFD establishes that the ecological status has to be determined by monitoring and assessing the so-called Biological Quality Elements (BQEs; Moss 2007, Lopez y Royo et al. 2011), which must be integrated into an index with the aim to detect temporal and spatial changes in the quality of water bodies (Bennett et al. 2011).

However, this innovativeness comes with a number of substantial challenges for ecologists in requiring complex and dynamic biological communities to be quantified into a single numeric score, for reference conditions to be established from which to measure the degree of change, and for this all to be carried out within a large number of water body types (Hering et al. 2010). The development of methods for water body quality assessment fulfilling the complex requirements of the WFD has been faced by each Member State individually (Søndergaard et al. 2005), resulting in the appearance of a wide variety of methods throughout Europe that differ greatly in the way of defining reference conditions, type vs. site-specific assessment, the number and nature of indices (metrics) used, etc. (Hering et al. 2010).

The workpackages of Module 4 of WISER Project (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery; www.wiser.eu) were conceived to evaluate the robustness and reliability of the different indices developed by the EU members prior the start of WISER as well as of those developed in collaboration with WISER. This is to be done mainly through quantification of pressure-indicator responses (see Deliverable 4.2-2) and uncertainty analysis, a powerful tool that allows the identification of the factors contributing to the potential misclassification of the ecological status class of water bodies (Clarke and Hering 2006, Staniszewski et al. 2006). The estimation of uncertainty is a central element in WFD-compliant assessment methods, since they are based on biological communities that show both spatial and temporal heterogeneity, and because errors will be introduced during sampling and analytical stages (Clarke and Hering 2006, Carstensen 2007, Kelly et al. 2009). If the major sources of variability are known, they can potentially be minimised through the re-design of sampling schemes (additional sampling sites or frequency), through improved training by operating procedures, CEN (European Committee for Standardization) guidance, taxonomic training or through the use of model-based assessment methods (Pont et al. 2009). For this reason, ecological status classification results should always be given in terms of probabilities depending upon the variability associated with these communities over time and space (Hering et al. 2010). However, only a small proportion of classification methods have put this into practice and the uncertainty analyses available in the literature are relatively scarce at the moment (but see Staniszewski et al. 2006, Kelly et al. 2009, Bennett et al. 2011).

The objectives of this deliverable are:

1. To summarize the characteristics (i.e. the target species, metrics used, definition of reference conditions, EQR calculation and classification boundaries, if pressure responses, and which pressures. have been tested) of the macroflora classification methods studied in WISER

2. To describe the new macroflora classification methods developed within WISER project

3. To determine which sources of variability (factors) associated with the sampling design different coastal Water Framework Directive monitoring programmes (implemented in Norway, Denmark, Bulgaria, Spain, Croatia, Slovenia, Cyprus, Greece and Portugal), encompassing 5 different classification methods based on macrophytes (either macroalgae or seagrasses), most greatly influence ecological status classifications of water bodies.

2. Description of methods studied in WISER to classify coastal water status with macrophytes

WISER consortium is using 7 different classification methods based on macrophyte metrics developed under the WFD to monitor the ecological quality of coastal water bodies in different regions of Europe. These indices and their corresponding regions and countries of application are: i) "Multi Species Maximum Depth Index" (MSMDI, North Atlantic - Norway), ii) "RSLA" (North Atlantic-Norway), iii) "Eelgrass Depth Limit" (EDL, Baltic Sea - Denmark), iv) "*Posidonia oceanica* Multivariate Index" (POMI, Mediterranean - Spain and Croatia), v) "Ecological Index" (EEI-c, Black Sea – Bulgaria; Mediterranean Sea- Greece, Cyprus, Slovenia), vi) "Marine Macroalgae Assessment Tool" (MarMAT, North Atlantic- Portugal) and vii) "Rocky Intertidal Community Quality Index" (RICQI, North Atlantic - Spain). The indices differed in their target macrophyte species, from a list of specific macroalgae (MSMDI) to a single seagrass species (POMI), as well as in the nature and number of metrics used. Thus, whereas some indices included one single metric (e.g. lower depth limit, EDL), others were calculated integrating a series of attributes spanning different levels of organization (e.g. physiological, morphological, population and community levels, POMI). In the multimetric indices, there are also differences in the method used to integrate the variables, from a sum of metrics (EEI-c) to ordination techniques to integrate the group of variables (Principal Component Analysis, POMI). Finally, one of the most important differences among indices is how the EQR range is split into the five quality status classes established by the WFD (bad/poor/moderate/good/high; Birk and Hering 2006). Whereas the EQR range is split into 5 equal classes in most of the indices (0.2/0.4/0.6/0.8 boundary class values for MSMDI, RSLA, MarMAT), some others present status classes of unequal wide due to particular methodological restrictions (EDL, POMI, RICQI and EEI-c). All relevant information regarding the 7 indices included in the present study is summarized in Table 1.

Most of these classification methods have been developed prior the onset of WISER project (Table 1). MarMAT, RICQI and RSLA, on the contrary, have been developed in collaboration with WISER project. A brief description of MarMAT and RICQI is included in the following section.

Table 1. Macrophyte-based classification indices examined. The target species, metrics used, definition of reference conditions, EQR calculation and class boundaries of each index are provided. The pressures to which the indices have been tested are indicated. If uncertainty components have been analysed the references of the manuscripts/publications are provided. It is indicated if the indices have been (fully/partly) developed during WISER. The references describing the indices are also provided.

Index	Country of application	Target species	Metric/s used	Definition of reference conditions	EQR calculation	Boundaries	Response to pressure (assessed /not assessed)	Uncertainty analyses	Developed during WISER (yes/no)	References
MSMDI Multi Species Maximum Depth Index	North Atlantic (Norway)	*Saccharina latissima* *Chondrus crispus* *Rhodomela confervoides* *Coccotylus truncata* *Phyllophora pseudoceranoides* *Halidrys siliquosa* *Delesseria sanguinea* *Phycodrys rubens* *Furcellaria lumbricalis*	Lower depth limit		Scoring points (1, 0.8 or 0.6) are giving if max. depth of species is larger than boundary. If max depth ≤ 0.6 boundary-score is 0.4. If species disappeared due to anthroprogenic reasons – score is 0.2. Mean score for all gives index value.	0.2 / 0.4 / 0.6 / 0.8	Total Nitrogen	Analysed by Mascaró et al (submitted)	Uncertainty analyses have been conducted by WISER. Index developed outside WISER.	Swedish Environmental Protection Agency, 2007
RSLA	North Atlantic (Norway)	Two lists of selected species (80 or 65 species) in different water types	Multi-metric; ESG1/2-ratios, richness, abundance of greens and	Each metric has its own reference condition based on least disturbed sites or near reference conditions datasets	For positive correlation between EQR-values and metric: $EQR = \{[(X-LCl_r)/Cl_w] \times B_w\} + LB_r$	0.2 / 0.4 / 0.6 / 0.8	Total Nitrogen, Nitrate, Nitrite, Total Phosphorous, Phosphat	Not analysed	Yes, partly with support from Norwegian authoritie	

			browns, % opportunitics, % greens, % reds, % browns.		For negative correlation: $EQR = UB_r - \{[(X-LCl_r)/Cl_w] \times LB_w\}$ X=Value, L=Lower, CL_r =Class range, Cl_w= Class width, B_w= EQR Band width, B_r = EQR Band range, U=Upper.		e		s.	
EDL Eelgrass Depth Limit	Baltic (Denmark)	*Zostera marina*	Lower depth limit	Historical data (~1880-1930; summarised in Krause-Jensen & Rasmussen 2009; preliminary analyses published in Krause-Jensen et al. 2005) The 90% percentile of historical data from the various sites is defined as the reference level (by the Danish Envionmental Authorities)	EQR=present depth limit (mean)/reference	0.25 / 0.5 / 0.74 / 0.9 Defined by the Danish Environmental Authorities	Assessed through spatial analyses (Nielsen et al. 2002, Carstensen & Krause-Jensen 2009, Krause-Jensen et al. 2011, Dromph et al. in prep. Fürhaupter et al . 2011) and through time-series	Uncertainty components analysed (Balsby et al. submitted; Mascaró et al submitted)	Uncertainty analyses and additional pressure-response analyses have been conducted during WISER	Danish Nature Agency 2010; Karup et al. 2011. Milestone 6 report

							analyses (Markager et al. 2010, Carstensen et al. in prep.)			
POMI *Posidonia oceanica* Multivariate Index	Mediterranean (Spain, Croatia)	*Posidonia oceanica*	Physiological, morphological, population (density) and community, integrated onto a single scale using Principal Component Analysis		$EQR'_x = (CI_x - CI_{worst}) / (CI_{optimal} - CI_{worst})$ $EQR_x = (EQR' + 0.11) / (1 + 0.10)$ where EQR' is the ecological quality ratio of site *x*, CI_x, CI_{worst} and $CI_{optimal}$ are the scores of site *x*, the worst reference site and the optimal reference site on the first component of the PCA respectively.	0.1 / 0.325 / 0.55 / 0.775		Uncertainty analysed (Bennet et al 2011, Mascaró et al in press, Mascaró et al submitted)	Uncertainty analyses have been conducted by WISER. Idex developed prior to WISER.	Romero et al., 2007
EEI-c (Ecological Evaluation Index; all existing for the index information)	Mediterranean (Greece, Cyprus, Slovenia, Bulgaria)	*Cymodocea nodosa*-ESG IA *Ruppia cirrhosa*-ESG IA *Cystoseira barbata*-ESG IB *Gracilaria bursa-pastoris*-ESG IIA *Cladophora* spp.-ESG IIB *Ulva* spp.-ESG IIB	Coverage (%) of 5 different Ecological Status Groups clustered hierarchically into two ESG's	Low pressure (MA Lusi <3) conditions that support benthic macrophyte communities of high diversity where *Cystoseira* or other perennial macroalgae species dominate (mean % coverage >60%) all the year around. Opportunistic macroalgae (epiphytes or not)	$p(x,y) = a + b*(x/100) + c*(x/100)^2 + d*(y/100) + e*(y/100)^2 + f*(x/100)*(y/100)$ where *x* is the score in ESG I, *y* is the score in ESG II and *a*, …, *f* are the coefficients of the hyperbola: a = 0.4680, b =	0.04 / 0.25 / 0.48 / 0.76	YES to MA-LUSI index, in which following pressures are included: a) Indirect pressures from	Uncertainty analysed (Mascaró et al submitted)	Uncertainty analyses have been conducted by WISER. Idex developed prior to WISER.	Orfanidis et al., 2011

				abundance remains low (mean % coverage <30%) all the year around. A more detailed view of Mediterranean rocky coasts reference conditions can be summarized as follows (from MEDGIG): 1.Macroalgal communities of high diversity should be dominated quantitatively by brown algae mainly of the order Fucales in high irradiance sites and red algae of the order Corallinales (or other sciaphilic species) in vertical cliffs. 2.Dense well-developed macroalgal communities thriving in the upper infralittoral zone with most characteristic species belonging	1.2088, c = - 0.3583 d = -1.1289, e = 0.5129, f = -0.1869		Corine database (Urban, Commercial & Industrial, Agriculture) b) Direct pressures (Sewage outfall, Mariculture, Sediment nutrient release, Freshwater inputs, Harbor) c) Background trophic status d) Confinement			

				to the genera *Cystoseira, Sargassum, Lithophyllum, Peyssonnelia, Corallina and Padina*. Other common species belong to the genera *Halopteris, Stypocaulon, Dictyota, Dictyopteris, Laurencia, Cladophora* and *Jania*. 3.In the shadow zones (exposed steep vertical cliffs) *Lithophyllum byssoides* develops, forming important organogenic structures (trottoir). In marine caves with scarce light conditions a sciaphilic vegetation of red and green algae is dominant. 4. Spatio-temporal variability of the community's composition and abundance affected by hard substrata availability, intense						

				and frequency of natural disturbances, e.g. hydrodynamism, grazing, by seasonal cycle of light period and intense, and by limiting factors like nutrients.						
MarMAT Marine Macroalgae Assessment Tool	North Atlantic (Portugal)	RTL (Reduced Taxa List) (see annex 1)	species richness; proportion of Chlorophyt a; number of Rhodophyt a; number of opportunist s / ESG I (ratio); proportion of opportunist s; shore description (see annex 2); coverage of opportunist s (%)	For each single metric a different reference condition was established (see annex 3). Reference conditions were set based on historical and monitoring data (Gaspar et al., 2011)	For each single metric is attributed a score corresponding to the measured conditions. These scores are summed up to estimate the total score value. This sum of scores is then divided by 36 (the maximum possible sum of scores)to obtain a 0 – 1 EQR. The value of each of the MarMAT metrics varied from 0 to 4; this range was divided into five intervals that corresponded to the five quality classes, in accordance with the Normative Definitions (annex V in WFD) (WFD, 2000/60/EC): bad - 0,	0.2 / 0.4 / 0.6 / 0.8 (see annex 3)	assessed	Not analysed	Yes (partially)	Neto et al., 2011

					poor - 1, moderate - 2, good - 3, and high - 4 (Annex 3)					
RICQI Rocky Intertidal Community Quality Index	North Atlantic (Spain)	Rocky Intertidal Communities	Similarity with Reference Communities (ESS) Presence of Cystoseira (PC) Morphologically complex algae (MCA) Algal species richness (Ra) Invertebrate species richness (Rf) Ratio of faunal cover to whole assemblage cover (Pf) Herbivores cover (Ch)			0.2 / 0.4 / 0.6 / 0.82		Uncertainty analysed (Mascaró et al submitted)	yes	Díez et al., 2012

			Suspensivores cover (Cs)							

2.1. Description of the classification methods for coastal waters using macrophytes developed with WISER

2.1.1. Marine Macroalgae Assessment Tool

Under the scope of the Water Framework Directive (WFD, 2000/60/EC), ecological reference conditions and quality states were established for the biological quality element of macroalgae. The work combined both historical and current monitoring data from northern Portuguese coastal waters (Portuguese 'A5 typology', matching the European 'NEA1 typology'). Representative intertidal rocky shore sites were selected to: (1) have a robust variability in macroalgal communities within the water body type and (2) cover a gradient of anthropogenic pressure to understand the communities' changes due to disturbance. First, a 'reduced taxa list' (RTL, Table 2) was developed by selecting and grouping macroalgal taxa common in the study area, in proportion to the naturally occurring macroalgal taxonomic groups (Chlorophyta, Phaeophyceae – Heterokontophyta and Rhodophyta) and comprising taxa that require a moderate level of taxonomical identification expertise. Second, based on the RTL, the metrics of composition, i.e., species richness; the number and proportion of Chlorophyta, Phaeophyceae and Rhodophyta; the number and proportion of opportunists; ecological status groups (ESG) I, and ESG II; the ratios ESG I/ESG II, the number of opportunists/ESG I and the number of opportunists/ESG II; plus an abundance metric coverage of opportunists were studied to understand their behaviour under different levels of disturbance. The thresholds between the five quality conditions ('high', 'good', 'moderate', 'poor' and 'bad', similar to those required by the WFD to calculate the ecological quality status, Table 3) were defined for the above macroalgal metrics in compliance with the WFD (Gaspar et al. 2011). Hence, reference conditions – pristine situations that exist or would exist with no or very minor disturbances from anthropogenic pressures – were established together, since this class of excellent quality is included in the 'high' quality condition.

Table 2 – Reduced Taxa List (RTL) for the A5 Portuguese Coastal Water (CW) typology (NEA 1 for Portuguese northern coast). ESG = Ecological Status Groups.

Reduced Taxa List: (CW A5 PT type)	**ESG**	**Opportunistic**
Chlorophyta:		
Bryopsis spp.	II	Yes
Other Filamentous Chlorophyta (1)	II	Yes
Cladophora spp.	II	Yes
Codium spp.	II	
Ulva spp. ('Sheet-type')/*Ulvaria obscura/Prasiola stipitata* (2)	II	Yes

Ulva spp. ('Tubular-type')/*Blidingia* spp. (3)	II	Yes

Phaeophyceae (Heterokontophyta):

Bifurcaria bifurcata	I	
Cladostephus spongiosus	I	
Colpomenia spp./*Leathesia marina*	II	
Cystoseira spp.	I	
Desmarestia ligulata	II	
Dictyopteris polypodioides	II	
Dictyota spp.	II	
Filamentous Phaeophyceae (4)	II	Yes
Fucus spp.	I	
Halopteris filicina/H. scoparia	II	
Himanthalia elongata	I	
Laminaria spp.	I	
Pelvetia canaliculata	I	
Ralfsia verrucosa	I	
Saccorhiza polyschides	I	

Rhodophyta:

Acrosorium ciliolatum/Callophyllis laciniata/Cryptopleura ramosa	II	
Ahnfeltia plicata	I	
Ahnfeltiopsis spp./*Gymnogongrus* spp.	II	
Apoglossum ruscifolium/Hypoglossum hypoglossoides	II	
Asparagopsis armata /Falkenbergia rufolanosa	II	
Bornetia spp./*Griffithsia* spp.	II	
Calliblepharis spp.	I	
Catenella caespitosa/Caulacanthus	II	

ustulatus		
Champiaceae (5)	II	
Chondracanthus acicularis	II	
Chondracanthus teedei	II	
Chondria spp.	II	
Chondrus crispus	I	
Calcareous encrusters (6)	I	
Calcareous erect (7)	I	
Dilsea carnosa/Schizymenia dubyi	II	
Gelidiales (8)	I	
Gigartina pistillata	II	
Gracilaria spp.	II	
Grateloupia filicina	II	
Halurus equisetifolius	II	
Hildenbrandia spp.	I	
Laurencia spp./*Osmundea* spp.	II	
Mastocarpus stellatus/Petrocelis cruenta	I	
Nitophyllum punctatum	II	
Other Filamentous Rhodophyta (9)	II	Yes
Phyllophora spp./Rhodymenia pseudopalmata	II	
Palmaria palmata	I	
Peyssonnelia spp.	I	
Plocamium cartilagineum/Sphaerococcus coronopifolius	I	
Porphyra spp.	II	Yes
Pterosiphonia complanata	II	
Scinaia furcellata	I	

1) *Chaetomorpha*, *Pseudendoclonium*, *Rhizoclonium*, Ulothricales. 2) *Ulva* spp. 'Sheet-type' in opposition to 3) 'Tubular-type' in the sense 'of 'ex- *Enteromorpha* spp.'. 4) Ectocarpales/*Sphacelaria* spp. 5) *Champia*, *Chylocladia*, *Gastroclonium*, *Lomentaria*. 6) *Lithophyllum*, *Melobesia*, *Mesophyllum*, *Phymatolithon*. 7) *Amphiroa*, *Corallina*, *Jania*. 8) *Gelidium*, *Pterocladiella*. 9) *Acrochaetium*, *Aglaothamnion*, *Antithamnion*, *Bangia*, *Boergeseniella*, *Brongniartella*, *Colaconema*, *Callithamnion*, *Ceramium*, *Compsothamnion*, *Dasya*, *Erythrotrichiaceae*, *Herposiphonia*,

Heterosiphonia, *Janczewskia*, *Leptosiphonia*, *Lophosiphonia*, *Ophidocladus*, *Pleonosporium*, *Plumaria*, *Polysiphonia*, *Pterosiphonia* (except *P. complanata*), *Pterothamnion*, *Ptilothamnion*, *Rhodothamniella, Streblocladia*, *Vertebrata*

Table 3 – Boundaries for each of the MarMAT metrics, sum of scores and EQR. Translation of the achieved EQR in the EQS (bad, poor, moderate, good or high) when assessing the ecological quality of rocky shores.

Metrics	Bad	Poor	Moderate	Good	High
Species richness (a)	0 - 6	7 - 13	14 - 20	21 - 27	28 - 54
Proportion of Cholophyta	0.32 - 1	0.27 - 0.31	0.21 - 0.26	0.15 - 0.20	0 - 0.14
Number of Rhodophyta	0 - 3	4 - 8	9 - 12	13 - 17	18 - 33
Number of opportunists / ESG I	≥1.23	1.01 - 1.22	0.80 - 1.00	0.58 - 0.79	<0.58
Proportion of opportunists	0.59 - 1	0.47 - 0.58	0.35 - 0.46	0.23 - 0.34	0 - 0.22
Coverage of oportunists (%) (a)	72 - 100	59 - 71	46 - 58	33 - 45	0 - 32
Shore description	-	15 - 18	12 - 14	8 - 11	1 - 7
Corresponding score to metrics class	0	1	2	3	4
Sum of scores	0 - 7	8 - 14	15 - 21	22 - 28	29 - 36
EQR	0 - 0.2	0.2 - 0.4	0.4 - 0.6	0.6 – 0.8	0.8 - 1
EQS	Bad	Poor	Moderate	Good	High

(a) factor of 2, counts twice in the metrics sum of scores calculation.

This classification method has been published in:

Neto, J.M., et al., Marine Macroalgae Assessment Tool (MarMAT) for intertidal rocky shores. Quality assessment under the scope of the European Water Framework Directive. Ecol. Indicat. (2011), doi:10.1016/j.ecolind.2011.09.006

A copy of this article is included in the Annex.

2.1.2. Rocky Intertidal Communities

The aim of this paper is to develop a new methodology for assessing the quality of coastal waters along the Atlantic Iberian coasts, based upon Basque coast rocky intertidal assemblages, compliant with the European Water Framework Directive (WFD, 2000/60/EC). Biological data collected over a 20-year period, during the gradual introduction of a sewerage plan, are compared to several reference stations in order to differentiate various degrees of community alteration. A quality index (RICQI: Rocky Intertidal Community Quality Index) is drawn up, on the basis of: indicator species abundance; morphologically complex algae cover; species richness; and faunal cover (herbivore and suspensivore cover, proportion of fauna with respect to the whole assemblage). An independent dataset collected in Plentzia Bay (Basque coast, N. Spain), before and after the set-up of a wastewater treatment plant, is used in order to validate RICQI. A conceptual model based on our results is proposed, which describes successional stages of assemblages along a gradient of increasing environmental disturbance and associated values of the metrics included in the index. The performance of this new approach is compared with that of the quality of rocky bottoms index (CFR, Juanes et al., 2008), used presently as the official method for assessing the ecological status of rocky assemblages inthe Atlantic coastal waters of Spain. Both indices respond to changes in community structure, associated with pollution removal. However, the RICQI index shows a more accurate response, identifying different degrees of disturbance.

This classification method has been published in:

Díez I, M. Bustamante, A. Santolaria, J. Tajadura, N. Muguerza, A. Borja, I. Muxika, J.I. Saiz-Salinas, J.M. Gorostiaga. 2012. Development of a tool for assessing the ecological quality status of intertidal coastal rocky assemblages, within Atlantic Iberian coasts. Ecological Indicators 12: 58–71

A copy of this article is included in the Annex.

3. Assessment of uncertainty of classification of coastal waters status using macrophyte indicators

We have quantified the uncertainty of classification of coastal waters status using macrophyte indicators and identified the components of monitoring programs that mostly contribute to the risk of misclassification. We first separately analysed the uncertainty of classification of coastal waters in monitoring programs using two macrophyte indicators (namely, "Maximum eelgrass depth limit" for Danish waters and "Posidonia multivariate index-POMI" for Catalan, Balearic and Croatian waters, see sections 3.1 and 3.2). We present a summary of the results on uncertainty assessments conducted for "Maximum eelgrass depth limit" and "Posidonia multivariate index-POMI" in sections 3.1 and 3.2 and include a copy of the respective articles/submitted manuscripts in the Annex. Then, we compared the magnitude of sources of uncertainty and the risk of misclassification across 5 macrophyte classification methods used in the WFD (see section 3.3). A detailed description of the uncertainty analyses conducted across classification methods is provided in section 3.3.

3.1. Sources of uncertainty associated with the monitoring of the "Maximum depth limit in eelgrass (*Zostera marina*)"

Based on a long-term marine monitoring program of eelgrass maximum depth limit in Danish coastal waters we estimated the uncertainty contribution of year, diver, transect and replicates for each water body. For all variables the uncertainty increased with the maximum depth limit, which suggested that eelgrass depth limits were more difficult to determine or less well defined at large depths. We used either a Spheric or a Gaussian function to describe the relation between uncertainty and the maximum depth limit for each variable. This parameterization of the depth specific uncertainty allowed estimation of the total variance, which can be used to evaluate survey designs. The total variance was compared with the time budget for a survey in a water body. If a maximum time limit was allocated to survey a water body, the surveys that resulted in the lowest variance of the maximum depth limit used 2 divers if 100 h were available and 3 divers if 200h were available, 2 or 3 years of survey and 4 to 8 transects.

The results of this study are provided in the submitted manuscript:

Thorsten J. S. Balsby, Jacob Carstensen, Dorte Krause-Jensen. Sources of uncertainty in estimation of eelgrass depth limits. Hydrobiologia (submitted).

A copy of this manuscript is included in the Annex.

3.2. Uncertainty of classification of coastal waters using "*Posidonia oceanica* multivariate index (POMI)".

3.2.1. Uncertainty of classification using POMI of Catalan coastal waters

We assessed the *Posidonia oceanica* multivariate index (POMI) bio-monitoring program for its robustness in classifying the ecological status of Catalan coastal waters (Spain, W Mediterranean) within the Water Framework Directive. We used a 7-year dataset, covering 30 sites along 500 km of the Catalan coastline to examine which version of POMI (14 or 9 metrics) maximizes precision in classifying the ecological status of meadows. Five factors (zones within a site, sites within a water body, depth, years and surveyors) that potentially generate classification uncertainty were examined in detail. Of these, depth was a major source of uncertainty, while all the remaining spatial and temporal factors displayed low variability. POMI 9 matched POMI 14 in all factors, and could effectively replace it in future monitoring programs.

This study has been published in:

BENNETT, S., ROCA, G., ROMERO, J., ALCOVERRO, T. (2011) Ecological status of seagrass ecosystems: An uncertainty analysis of meadow classification based on the Posidonia multivariate index (POMI). Marine Pollution Bulletin: 62: 1616-1621.

A copy of this article is included in the Annex.

3.2.1. Sources of uncertainty and quantification of the risk of misclassification of Catalan, Balearic and Croatia coastal waters using POMI

Uncertainty analyses allow the identification and quantification of the factors that contribute to the potential misclassification of the ecological status of water bodies, helping to improve the sampling design used in monitoring. Here we used a Posidonia oceanica multivariate index (POMI) biomonitoring dataset covering a total of 81 sites distributed throughout 28 water bodies from the coast of Catalonia, Balearic Islands and Croatia to determine the levels of uncertainty associated with each region and how they change according to the quality status of water bodies. Overall, variability among sites (meadows) within water bodies was the factor that generated the greatest risk of misclassification among the three regions, within which the Balearic Islands had the lowest uncertainty, followed by Croatia and Catalonia. When water bodies classified in good/high quality were separated from those in moderate/poor status classes, we found that the latter displayed higher levels of uncertainty than the former.

The results of this study are in this submitted manuscript:

MASCARO, O., BENNETT, S., MARBA, N., NIKOLIC, V., ROMERO, J., DUARTE, C.M., ALCOVERRO, T.. Uncertainty analysis along the ecological quality status of water bodies: the response of the *Posidonia oceanica* multivariate index (POMI) in three Mediterranean regions. Marine Pollution Bulletin. (submitted)

A copy of this manuscript is included in the Annex.

3.3. Exploring the robustness of different macrophyte-based classification methods to assess the ecological status of coastal and transitional ecosystems under the WFD

We compiled extensive bio-monitoring data from several macrophyte-based classification methods developed by different EU Members, which include data addressing spatial, temporal and human-induced sources of variability, to identify, through the application of uncertainty analysis, the major sources of uncertainty for coastal water classification. This exercise should help to design monitoring programs that minimise the risk of misclassification.

The analyses are be based on EQR datasets of either official or non-official bio-monitoring programmes of the different indices from which a data set including enough temporal and spatial replication was available, and the factors analysed will include spatial scales of sampling (variability among zones within a site, among sites within a water body, variability among regions and variability among depths), the temporal scale of sampling (variability among years) and the human-associated source of error (variability between surveyors). These factors represent some the key sources of variability associated with the design and implementation of a bio-monitoring program, and highlight how certain elements of a sampling design can influence the reliability and robustness of the ecological status classification of coastal water bodies. With this approach, we try to gain insight into the current status of these methodologies proposed for European waters under the WFD and detect their main weaknesses to provide robust foundation for monitoring as well as guide decision in management plans.

3.3.1. Methods

- 3.3.1.1. Methods of classification examined

We compared the magnitude of the sources of uncertainty and the risk of misclassification of European coastal waters using the following methods of classification:

- Multi Species Maximum Depth Index (MSMDI)
- Eelgrass Depth Limit (EDL)
- Posidonia multivariate Index (POMI)
- Ecological Evaluation Index coastal (EEI-c)
- Rocky Intertidal Community Quality Index (RICQI)

The characteristics of these methods of classification are described in section 2 and summarised in Table 1.

- 3.3.1.2. Variance extraction

In the current study, the factors examined that potentially contribute to the uncertainty of the EQR estimations of coastal water bodies differ greatly among the 5 indices, especially due to differences in both the metrics used and their corresponding spatial and temporal sampling designs (Table 4). The total variance and variance components associated to each factor were estimated for all indices using a linear mixed effects model in the lme4 package of R (Bates 2005 and 2007, Version 2.10.1, R_Development_Core_Team 2009). When sufficient data was available, factors were treated as random intercepts, either nested or crossed depending on the index (Table 4). Note that the variability among water bodies, whilst important in the analysis of variance components, is not discussed in this study because by definition they should differ in their ecological status. Variance components were determined by calculating the proportion of the total variance (σ^2_T) explained by each individual factor. Thus, total variance in mean EQR values for each index was given by the sum of variances associated to each of the factors included in the model (σ^2_X) plus the variance not explained by the model (σ^2_R; Table 4). The proportion of total variance (P_{samp}; Table 4) explained by each factor was given by the equation, following Clarke et al. (2006):

$$P_{samp} = 100\ \sigma^2_X / \sigma^2_T \quad (1)$$

Posteriorly for each index, the extracted variances were grouped into four main sources of uncertainty: i) temporal scale of sampling (variability among years), ii) spatial scale of sampling (including variability among zones within a site, among sites within a water body, variability among regions, variability among depths, etc.), iii) human-associated source of error (variability among surveyors) and iv) the residual term of the analysis (the variance in mean EQR values not explained by the model) in order to allow a further comparison of the results among indices that would help drawing general conclusions about these macrophyte-based classification methods (see Table 4).

All data satisfied the assumption of homogeneity of variance based on plots of the residuals against the fitted EQR values; therefore, no transformation of the data took place.

- 3.3.1.3. Uncertainty analysis

Having calculated the variation in mean EQR scores for all factors within each index, the uncertainty in ecological status classification was estimated using WISERBUGS (WISER Bioassessment Uncertainty Guidance Software®, Clarke 2010). WISERBUGS helps determine whether an observed ecological status classification is indeed the most probable classification for a particular site, given the inherent sources of variability. WISERBUGS sums the observed value for a given site with a random standard normal deviate, of the known SD, with a mean of zero (Clarke and Hering 2006). It repeats this simulation 10^4 times to produce a frequency distribution of possible EQR values for the particular site or water body. The simulated EQR values are grouped into their corresponding status classes, from which the probability of misclassifying the original observed value can be determined. Because the current study was interested in the uncertainty in classification generated by a particular factor (rather than the probability of misclassifying individual sites), the simulation was repeated for the full range of possible observed EQR values (0 - 1).

Table 4. Factors of the different groups included in the main sources of uncertainty, and the variance components.

Index	Main sources of uncertainty			Variance components
	Temporal scale	Spatial scale	Human-associated error	
MSMDI Multi Species Maximum Depth Index	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si})	· Surveyor (σ^2_{Su})	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_{Sur} + \sigma^2_R$
EDL Eelgrass Depth Limit	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_R$
POMI *Posidonia oceanica* Multivariate Index	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si}) · Zone:(Site:Water Body:Region) (σ^2_Z) · Depth (σ^2_D)	· Surveyor (σ^2_{Su})	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_Z + \sigma^2_D + \sigma^2_{Su} + \sigma^2_R$
RICQI Rocky Intertidal Community Quality Index	· Year (σ^2_Y)	·Site:Water Body (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Si} + \sigma^2_R$
EEI-c Ecological Evaluation Index	-	· Water Body (σ^2_{WB}) · Site:Water Body (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_R$

3.3.2. Results

- 3.3.2.1. Analysis of the uncertainty associated to the ecological status classification

Depending on the index, the factors examined displayed different levels of uncertainty in the ecological status classification of water bodies. Generally for all factors, the probability of misclassification peaks when a site's observed EQR score is very close to the boundary between two status classes, usually around 50%. In contrast, when the observed EQR falls in the middle of a status class the probability of misclassification declines to the minimum. Probabilities of misclassification >50% may indicate that the associated variability is actually higher than the EQR range of the status class. The magnitude of these maximum and minimum uncertainty levels differ greatly among factors and indices as a result of the differences in the variance extracted. In summary, the higher the variability, the higher its probability of misclassification even in the centre of the status class ranges.

i. Multi Species Maximum Depth Index (MSMDI)

In this index, all the examined factors showed a low variability in the mean EQR scores, which determined also low associated probabilities of misclassification. On the one hand, the factors “year”, “region” and “surveyor” displayed almost negligible levels of variability, explaining only 2.2%, 0.0% and 4.0% of total variance respectively (Table 5). This corresponded to a minimum probability of misclassification of 0% and maximum of 50% for each of these factors (Fig. 1). Even still low, variability in the mean EQR scores among different sites was higher, explaining up to 24.4% of total variance (Table 5) and resulting in levels of uncertainty ranging from 6% to 50% (Fig. 1). Finally, the variability not explained by the model represented up to 27.6% of total variance, for which the levels of uncertainty associated to unknown sources ranged from 7% to 50% (Table 5, Fig. 1).

Table 5. MSMDI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of five random effects. Colon between factors represents nesting (i.e. Site:WB signifies that site is nested within water body).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	21	Crossed	0.016156	0.000261	2
Region	(Intercept)	2	Crossed	0.000000	0.000000	0
Water Body	(Intercept)	12	Crossed	0.071020	0.005044	42
Site:WB	(Intercept)	20	Nested	0.054312	0.002950	24
Surveyor	(Intercept)	4	Crossed	0.021960	0.000482	4
Residual				0.057723	0.003332	28

ii. Eelgrass Depth Limit (EDL)

All factors analysed for this index showed relatively high variability, determining also high probabilities of misclassification. In this case, however, the levels of uncertainty associated to each factor increase along the EQR range as the width of the status classes narrows (0.25/0.5/0.74/0.9 boundary values; Fig. 2). The factor “year” displayed the lowest levels of

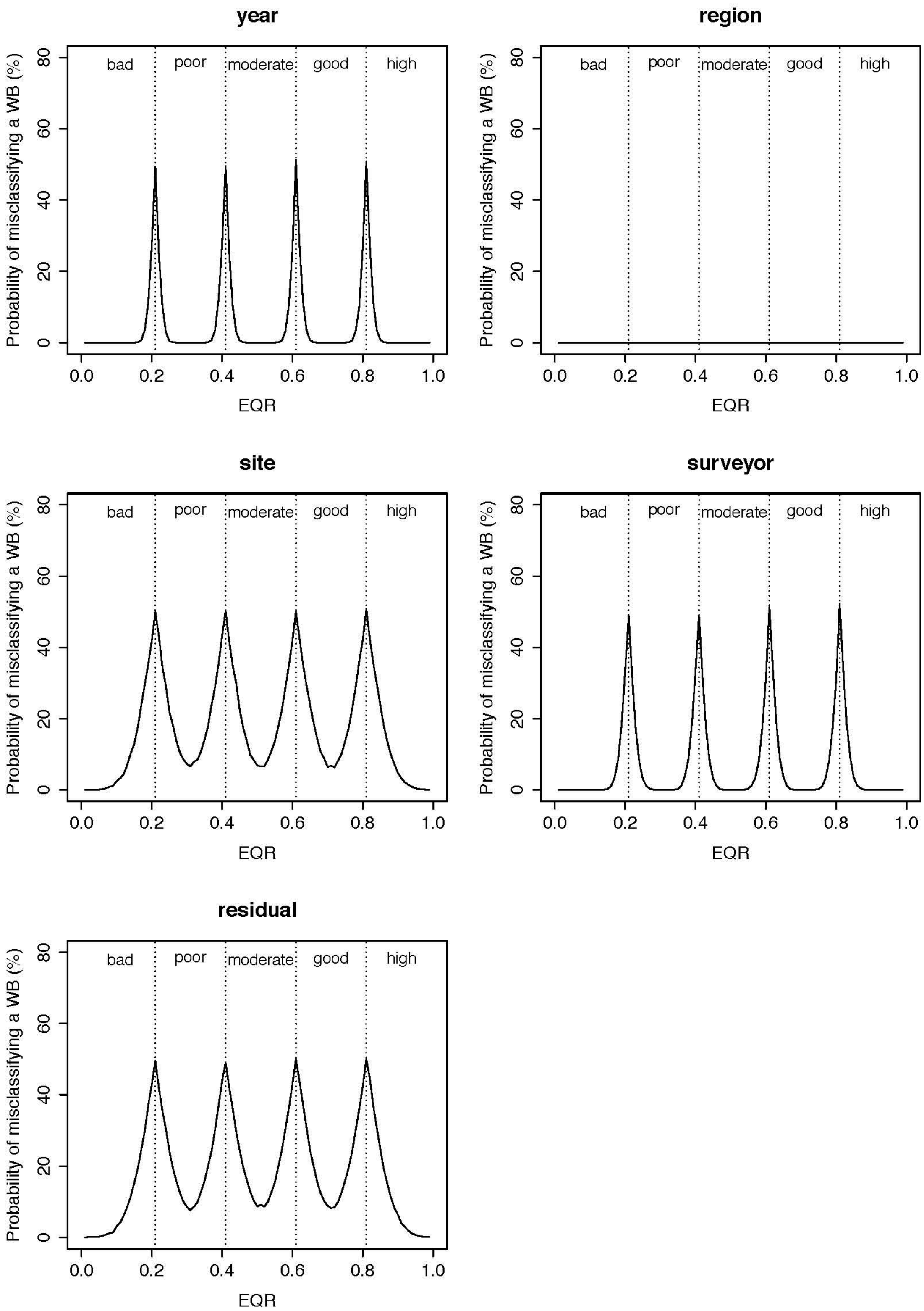

Figure 1: *Probability of misclassifying the ecological status class associated to the different factors analysed for MSMDI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.*

variability in the mean EQR scores, representing 9.7% of total variability (Table 6). Its corresponding probabilities of misclassification included minimum values from 16% to 36% and maximum of 50% to 54%, following the EQR range (from 0 to 1; Fig. 2). The factors "region" and "site" showed a higher and similar variability in the mean EQR scores observed, explaining 30.2% and 24.4% of total variance respectively (Table 6). As a result, the probability of misclassification in the centre of a status class ranged from 40% to 58% along the EQR range (from 0 to 1), whilst in the boundary between two status classes ranged from 54% to 64% (approximate values for the two factors; Fig. 2). For the residual term of the analysis, it represented up to 30.4% of total variance, for which high levels of uncertainty were associated to unknown factors for this index (minimum levels from 42% to 60% and maximum from 60% to 65% along the EQR range; Fig. 2).

Table 6. EDL results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	23	Crossed	0.088461	0.007825	10
Region	(Intercept)	9	Crossed	0.155929	0.024314	30
Water Body:Region	(Intercept)	20	Nested	0.068029	0.004628	6
Site:(WB:Region)	(Intercept)	254	Nested	0.139132	0.019358	24
Residual				0.156419	0.024467	30

iii. Posidonia oceanica Multivariate Index (POMI)

In this index, great differences in the variance and the associated risk of misclassification were observed among the several analysed factors. On the one hand, the factors "year", "site", "zone" and "surveyor" displayed very low variability, representing only 4.9%, 4.5%, 3.0% and 0% of total variance each (Table 7). As a result, their associated probability of misclassification was also low, ranging from minimum levels of 2.6%, 1.9% and 0.4% for "year", "site" and "zone" respectively, to maximum levels of c.a. ≤50%; since the variance of the factor "surveyor" was negligible (σ^2<0.000000), the uncertainty associated to this factor was considered 0% along the whole EQR range (Fig. 3). On the other hand, the highest variability was observed in the mean EQR scores among regions and depths, which explained 29.8% and 25.8% of total variance respectively (Table 7). This corresponded with an also high probability of misclassification associated to these factors, from minimum values of 36% and 33% to maximum of 54% and 53% for "region" and "depth" respectively (Fig. 3). The residual term of the analysis represented up to 17.1% of total variance, determining relatively high levels of uncertainty due to unknown factors (from 24% to ≤50%; Fig. 7).

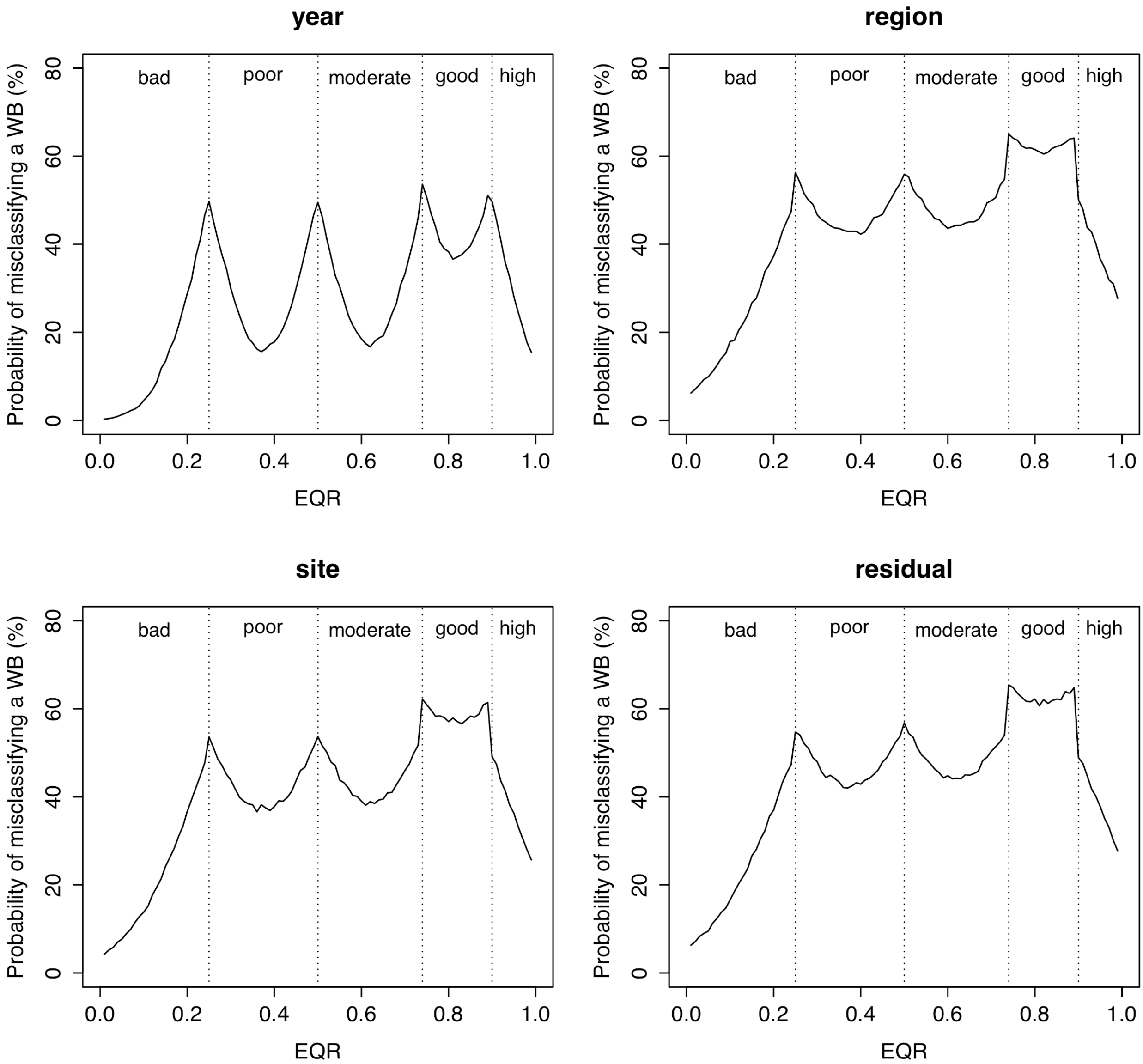

Figure 2: *Probability of misclassifying the ecological status class associated to the different factors analysed for EDL. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.25; Poor = 0.26 – 0.5; Moderate = 0.51 – 0.74; Good = 0.75-0.9 and High = 0.91 – 1.*

Table 7. POMI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of seven random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	6	Crossed	0.050508	0.002551	5
Region	(Intercept)	3	Crossed	0.125150	0.015663	30
Water Body:Region	(Intercept)	50	Nested	0.088485	0.007830	15
Site:(WB:Region)	(Intercept)	103	Nested	0.048587	0.002361	4
Zone:(Site:WB:Region)	(Intercept)	119	Nested	0.039436	0.001555	3
Depth	(Intercept)	2	Crossed	0.116480	0.013568	26
Surveyor	(Intercept)	4	Crossed	0.000001	0.000000	0
Residual				0.094870	0.009000	17

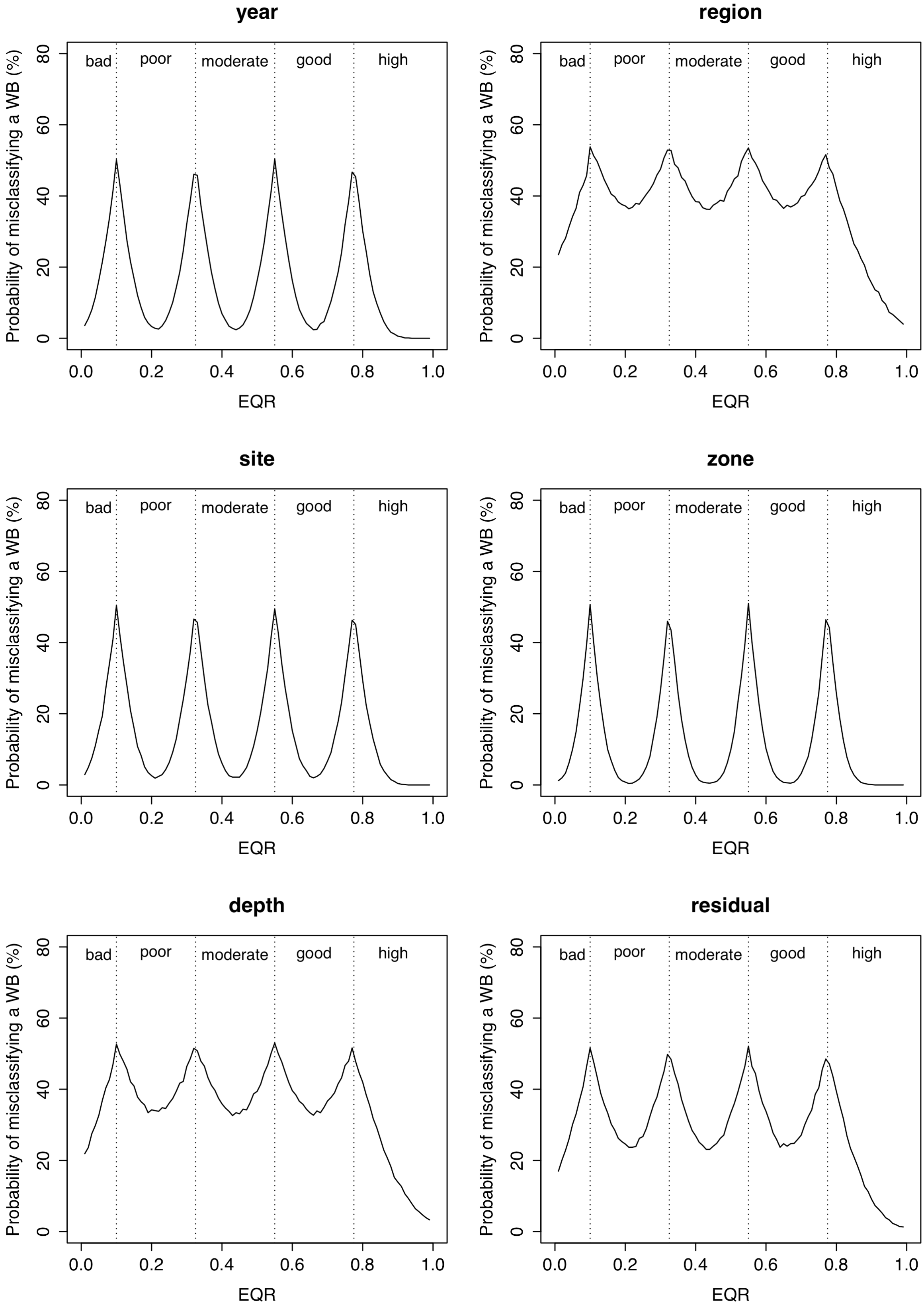

Figure 3: *Probability of misclassifying the ecological status class associated to the different factors analysed for POMI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.09; Poor = 0.1 – 0.324; Moderate = 0.325 – 0.549; Good = 0.550-0.774 and High = 0.775 – 1.*

iv. Rocky Intertidal Community Quality Index (RICQI)

In this index, the lack of replication among different water bodies may determine the high variability associated to the factors included in the biomonitoring program. On the one hand, variability among years was relatively high, representing 14.4% of total variance (Table 8) and determining levels of uncertainty that ranged from 17% to 50% (Fig. 4). On the other hand, variance associated to the spatial factor "site" was extremely high, representing 73% of total variance (Table 8) and displaying uncertainty levels between 54% and 61% along the whole EQR range (Fig. 4). Finally, the residual term of the analysis accounted for 12.6% of total variance (Table 8), and with uncertainty levels that ranged from 14% to 50% (Fig. 4).

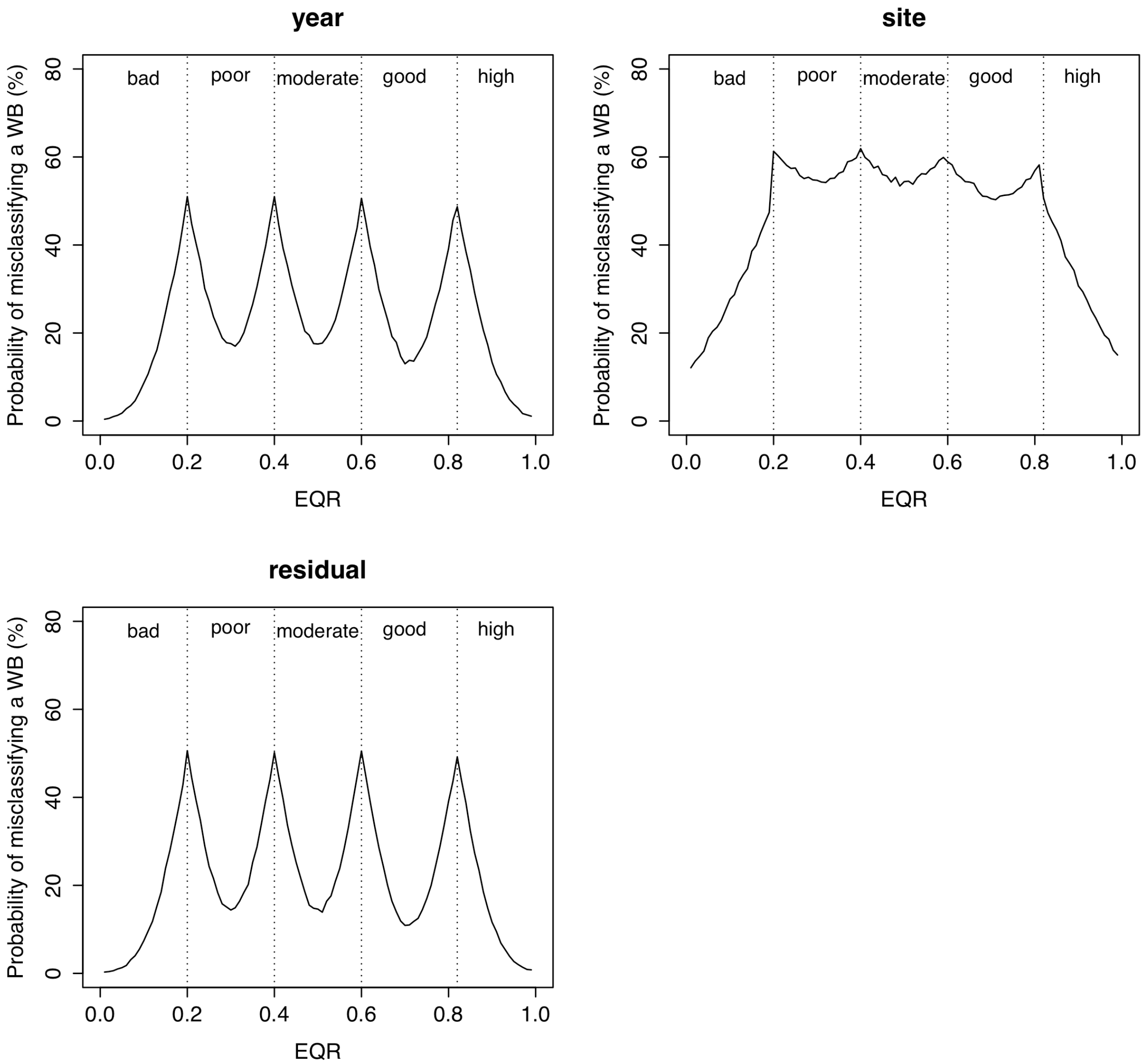

Figure 4: *Probability of misclassifying the ecological status class associated to the different factors analysed for RICQI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.82 and High = 0.83 – 1*

Table 8. RICQI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	3	Crossed	0.073332	0.005378	14
Site	(Intercept)	7	Crossed	0.164836	0.027171	73
Residual				0.068418	0.004681	13

v. Ecological Evaluation Index (EEI-c)

In this index, variability among sites was negligible (σ^2<0.000000; Table 9), for which the risk of misclassification associated to this factor was 0% along the whole EQR range (Fig. 5). In contrast, the residual variance in mean EQR values was high, accounting for 30.5% of total variance (Table 9) and determining high levels of uncertainty that remained ≥50% almost along the whole EQR range (Fig. 5). The increasing width of the status classes along the EQR range (from 0 to 1) promoted that the general risk of misclassification decreased from "poor" to "high" status.

Table 9. EEI-c results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of three random effects. Colon between factors represents nesting (i.e. Replicate:(Site:WB) signifies that replicate is nested within site that, at the same time, is nested within WB).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Water Body	(Intercept)	4	Crossed	0.292036	0.085285	68
Site:WB	(Intercept)	6	Nested	0.000006	0.000000	0
Replicate:(Site:WB)	(Intercept)	18	Nested	0.086976	0.007565	6
Residual				0.179911	0.032368	26

- 3.3.2.2. Main common sources of uncertainty among indices

For each index, the variances extracted for the different factors were grouped into four main sources of uncertainty: i) the temporal scale of sampling (variability among years), ii) the spatial scale of sampling (including variability among zones within a site, among sites within a water body, variability among regions, variability among depths, etc.), iii) human-associated sources of error (variability among surveyors) and iv) the residual term of the analysis (the variance in mean EQR values not explained by the model).

The spatial scale of sampling (excluding variability among water bodies) represented the main source of uncertainty, accounting for an average proportion of 43±15 % of total variance among the different indices (mean±SE; see Table 10). However, the factors grouped in this category and their associated variability differed greatly among the indices. Another important general source of uncertainty is the residual variance of the model, which accounted for an average of 24±4 % (in mean±SE; see Table 10,) of the total

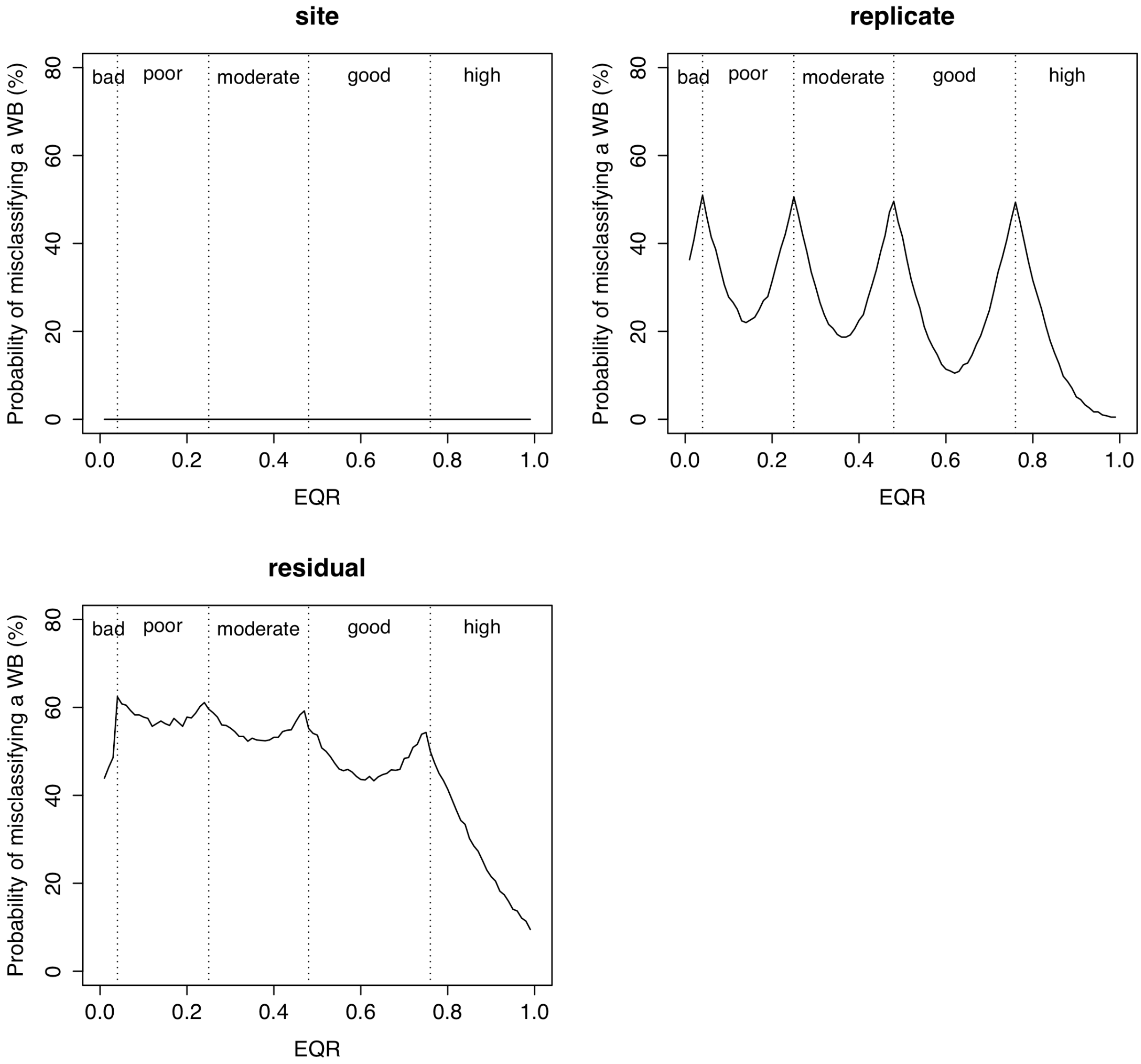

Figure 5: *Probability of misclassifying the ecological status class associated to the different factors analysed for EEI-c. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.*

variability among the different indices. In contrast, our results show that neither the temporal scale of sampling nor the human-associated source of error are important sources of uncertainty when classifying the ecological status of water bodies, as indicated by the low proportion of the total variance explained by the factors “year” and "surveyor" in the indices in which they were measured (8±3% and 2±3% respectively in mean±SE; see Table 10).

Table 10. Proportion of the total variance (in %) explained by the different factors grouped in the main sources of uncertainty for each index, excluding WB.

Index	Main sources of uncertainty			
	Temporal scale	Spatial scale	Human-associated error	Residual
MSMDI Multi Species Maximum Depth Index	2	24	4	28
EDL Eelgrass Depth Limit	10	54	-	30
POMI *Posidonia oceanica* Multivariate Index	5	63	0	17
RICQI Rocky Intertidal Community Quality Index	14	73	-	13
EEI-c Ecological Evaluation Index	-	0	-	30
mean	8	43	2	24
SE	3	15	3	4

3.3.3. Discussion

Including uncertainty estimation into assessment schemes is a major challenge of the next phase of WFD implementation (Hering et al. 2010). Even though the underlying statistical principles are relatively simple and appropriate tools for uncertainty estimation are available (e.g. Clarke and Hering 2006, Carstensen 2007), data addressing the individual sources of error are still needed, such as temporal and spatial variation of sampling, as well as differences between surveyors. This study is one of the first ones in which uncertainty analyses have been applied to several marine macrophyte based indexes, bringing some light to adequate designs in order to assess the ecological status of water bodies. Our results reveal that when analysing macrophyte communities, the factors related to the spatial scale of sampling added the highest levels of uncertainty whilst temporal variation and variability among surveyors were low. In addition, the residual term of the analysis added relatively high levels of uncertainty to the water body status classification of most indices, indicating that there are still unknown sources of variability that must be captured within the monitoring programmes.

Spatial variability has always been observed in natural communities, which becomes an important constrain when up scaling natural processes (Landres et al. 1999). In this study, the high levels of uncertainty associated to this factor are not surprising and may be related to the already observed high horizontal and vertical heterogeneity displayed by macrophyte communities (Ballesteros et al. 2007). Vertical variability has been attributed to factors associated to light attenuation with depth (Duarte 1991) and to the low rates of herbivory in deep sites compared to shallow depths (Tomas et al. 2005, Prado et al. 2007, Korpinen et al. 2007). All those natural processes, independent of any anthropogenic disturbances, influence structural and physiological parameters of macrophyte communities (Martínez-Crego et al. 2008), for which sampling at multiple depths result in highly variable EQR scores (from 25% to 37% of total variance in POMI and EI respectively). To reduce the risk of misclassification when assessing the ecological status of macrophyte communities, a relatively easy solution is that depth should remain fixed or be controlled in monitoring programs (see also Bennett et al. 2011). On the other hand, horizontal variability has been attributed to several factors acting from local (i.e. nutrient availability, sediment redox potential; Alcoverro et al. 1995) to regional scales (i.e. light, temperature; Marbà et al. 1996) that again influence structural and physiological parameters (Martínez-Crego et al. 2008). To capture this horizontal heterogeneity, bio-monitoring programmes must include sampling at different spatial scales, providing robust estimates of the ecological quality status classification at the water body level that include as much of this variability as possible, thereby minimizing the risk of misclassification (Kelly et al. 2009, Bennett et al. 2011). Even though bio-monitoring programmes from the different indices include sampling at several sites within each water body, only few of them include additional scales of replication below this level (POMI), resulting in a generally high uncertainty associated to the "site" factor (MSMDI, EDL, EI, RICQI). In these indices, it is strongly recommended to increase the sampling effort by adding a larger number of sites and within them, collecting different samples and averaging the metric values to provide robust estimates and minimize their associated risk of misclassification. This greater sampling effort may substantially increase the time and expense of the monitoring programmes, although it can be partially solved by maintaining the same number of replicates but just modifying the spatial sampling design to achieve a balance between financial constrains and a desirable index reliability. At a broader spatial scale, high variability among regions may indicate that they are separating groups of water bodies of similar ecological quality status. However, since this variability is above the scale of water body, at which the quality status is measured in the WFD, the risk of misclassification does not need to be minimized but included in the model and take into consideration when interpreting the uncertainty analysis results.

For the remaining factors, the uncertainty surrounding estimates in ecological status classification was very low within water bodies. Especially surprising is the case of inter-annual variability, which represented only between 1% and 9.7% of total variance depending on the index. As also reported by Bennett et al. (2011), this signifies that the EQR scores of water bodies are fairly consistent throughout the years, for which the frequency of sampling could be increased without greatly reducing the precision of ecological status estimates. Also surprising is the low variability among surveyors, which accounted only from 0% to 4% of total variance (for POMI and MSDMI respectively). This may be attributed to the fact that these particular macrophyte-based indices do not require complicated taxonomic identifications, which can greatly affect the precision of the EQR estimations in the case of other classification methods based on diatoms (Prygiel et al. 2002, Kelly et al. 2009) or freshwater macrophyte communities (Staniszewski et al. 2006). Finally, the residual term of the analysis represents all the variance that cannot be attributed to any of the factors included in the model, giving an idea of the accuracy of our approximation. In our study, it represented a relatively large proportion of total variance among the different indices (24±4% in mean±SE), indicating that other unknown sources of uncertainty may be affecting the ecological status classification of water bodies. In order to keep this variance to the minimum, further data concerning other factors related to the sampling design may need to be collected in those indices where it is relatively large (spatial variance, temporal variance, variance among surveyors, etc.).

Furthermore, our results showed that the risk of misclassifying the quality status of water bodies is also affected by the width of the status class in which the EQR score falls, as reported in Kelly et al. (2009), with narrower classes leading to greater probabilities of misclassification. Thus, indices in which the EQR range is not equally split into the 5 official classes present, for a certain variance associated to a factor, different uncertainty levels depending on the status class (see EDL, POMI, RICQI and EEI-c). This fact have drastic implications for bio-monitoring programs, because a greater sampling effort may need to be assigned to water bodies whose EQR score falls within the narrower status classes in order to reduce their associated variability and increase the confidence of the classification.

3.3.4. Conclusions

In summary, our study confirmed that the analysis of the uncertainty associated to the ecological quality status classification of water bodies are a good proxy to identify and quantify the factors that may affect the risk of misclassification. When applied to macrophyte monitoring programs, we have observed that the main sources of uncertainty are mostly associated to the sampling spatial scales, while temporal or human-induced errors seem to be less relevant. As a guide for taking management decisions, adequate sampling designs that include replication at different spatial scales within water bodies may substantially reduce this uncertainty. In some cases, it is not increasing the sampling effort but distributing it more efficiently within the allocated time and budget constrains that we will be able to maximize the confidence of estimations when assessing ecosystem health under the WFD.

The results of this study are in this submitted manuscript:

MASCARO, O., ALCOVERRO, T., DENCHEVA, K., KRAUSE-JENSEN, D., MARBÀ, N., MUXIKA, I., NETO, J., NIKOLIC, V., ORFANIDIS, S., PEDERSEN, A., PEREZ, M., ROMERO, J. Exploring the robustness of different macrophyte-based classification methods to assess the ecological status of coastal and transitional ecosystems under the WFD. Hydrobiologia (submitted).

A copy of this manuscript is included in the Annex.

4. Recommendations

The identification of the major sources of uncertainty of classification of coastal European waters using macrophyte indices helps improving the WFD monitoring programs in order to minimise the risk of misclassification of water bodies. According with our results:

- Horizontal spatial heterogeneity must be captured by sampling at different scales, providing robust estimates of the ecological quality status classification at the water body level that minimize the risk of misclassification.

- When using indices where water depth is not a component of it, depth should remain fixed or be controlled in monitoring programs in order to minimise vertical heterogeneity.
- Those indices where the distance between boundary classes is not uniform across the EQR range may need to assign a greater sampling effort to water bodies whose EQR score falls within the narrower status classes, in order to reduce their associated variability and increase the confidence of the classification. In contrast, sampling frequency has little effect on the precision of ecological status estimates.
- A greater replication effort should be assigned to those water bodies classified in moderate/poor/bad status, in order to capture the extra spatial variability coming from the effects of human pressures. On the other hand, it may be also a first warning that the spatial extent of water bodies may need to be redefined when differences in mean EQR values among different meadows of the same water body are excessively high, since an adequate spatial replication design will not be able to reduce the uncertainty associated to the classification system. A redefinition of the spatial extent and number of water bodies is strongly recommended in such cases.

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6. Annex

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Marine Macroalgae Assessment Tool (MarMAT) for intertidal rocky shores. Quality assessment under the scope of the European Water Framework Directive

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ABSTRACT

The *Water Framework Directive* (WFD) requires European Member States to assess the Ecological Quality Status (EQS) of their water bodies based on Biological Quality Elements (BQEs). A tool called MarMAT (*Marine Macroalgae Assessment Tool*) was developed to implement the WFD in Portugal, which assesses the EQS of Portugal's coastal intertidal rocky shores. MarMAT is a multimetric method that is compliant with the European WFD requirement. It is based on the composition (Chlorophyta, Phaeophyceae and Rhodophyta) and abundance (coverage of opportunists) of marine macroalgae. This study focused on the demands of the WFD to have the assessment methodologies legally accepted by the European Commission. The following factors were examined: (a) the response of MarMAT against anthropogenic pressures; (b) the ability of MarMAT to report all of the five quality classes (bad, poor, moderate, good and high); and (c) the performance of MarMAT, specifically in comparing the RSL (Reduced Species List) methodology with the utility of including the abundance (coverage of opportunists) metric and the necessity of locally adapted reference conditions and boundaries. MarMAT was high inversely correlated ($p < 0.001$) with anthropogenic pressure. MarMAT also successfully reported all of the quality classes (bad to high) and captured the community changes more accurately when using the coverage of opportunists metric. Because MarMAT satisfactorily covered all of the issues examined, MarMAT may be accepted as a compliant assessment methodology in the scope of the WFD requirements.

1. Introduction

The eutrophication of coastal systems as a result of anthropogenic activities is recognised worldwide as a major pollution threat (Norkko and Bonsdorff, 1996; Valiela et al., 1997; Raffaelli et al., 1998; Sfriso et al., 2001). Frequently, one of the main problems affecting these areas is a spatial shift in primary producers, which often prevails also in time. Undisturbed systems with low nutrient loadings are regularly dominated by slow-growing vegetation (e.g., *Zostera* sp. and *Fucus* sp.), while disturbed systems with enhanced nutrient loadings favour the growth of phytoplankton and opportunistic macroalgae (e.g., *Ulva* sp. and *Porphyra* sp.) (Raffaelli et al., 1998). Nutrients may arrive in the system as water is dissolved or as loose mats decompose after they have been accumulated (Raffaelli et al., 1998). Changes in the composition of primary producers can also lead to changes in associated communities (e.g., macroinvertebrates, fish, and shorebirds) (Raffaelli et al., 1998) and to changes in the materials and services these areas supply to surrounding environments (Jonge et al., 2000). Many management schemes implemented in the past few decades have sought to manage the physicochemical conditions of the water and sediment. These schemes were implemented to reduce the external nutrient loading of coastal systems, but the effective control of its efficiency has only recently been regarded as reasonable, with the implementation of monitoring programmes focused on the ecological integrity of aquatic systems. These programmes correspond to the implementation of recent water policies, such as the European Water Framework Directive (WFD, 2000/60/EC) or the USA's Clean Water Act (CWA, 2002/P.L. 107-303/USA).

The environmental objective of the WFD is to achieve a 'good water status' for surface and groundwater by 2015 and to prevent its deterioration in subsequent years throughout the Europe (WFD, 2000/60/EC) (see Mostert, 2003; Borja, 2005). The WFD requires European Union (EU) Member States (MS) to assess their surface water status by determining each water body's ecological and chemical status (WFD, 2000/60/EC). To assess the ecological quality based on the Biological Quality Elements (BQEs) the reference conditions (undisturbed or nearly so) must be defined, and the deviation of a given system to the conditions that can be measured at any other moment must be estimated. The difference in the quality observed between measurements and the reference conditions is called the Ecological Quality Ratio (EQR), and its values range from 0 (low quality) to 1 (high quality). The EQR is converted into the Ecological Quality Status (EQS); the assessment

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results are expressed as bad, poor, moderate, good, or high (detailed in the Common Implementation Strategy (CIS) documents: WFD CIS, 2003a,b,c,d).

The BQEs outlined by the WFD to assess Coastal Waters (CWs) include phytoplankton, benthic macroinvertebrates, and other aquatic flora, such as macroalgae and angiosperms (WFD, 2000/60/EC). Macroalgae are useful indicators of environmental quality because they can integrate environmental pressures, and they can respond to toxic substances, changes in nutrient concentrations and hydromorphology (Benedetti-Cecchi et al., 2001; Soltan et al., 2001; Panayotidis et al., 2004; Melville and Pulkownik, 2006; Yuksek et al., 2006; Arévalo et al., 2007; Scanlan et al., 2007; Krause-Jensen et al., 2008). These environmental alterations can be quantified through different measurable attributes (metrics), which individually or in combination, can be used to monitor the functioning of aquatic systems and infer their ecological status (Schramm, 1999; Orfanidis et al., 2001, 2003, 2011; Krause-Jensen et al., 2007; Scanlan et al., 2007; Wells et al., 2007; Juanes et al., 2008).

The reference conditions defined for composition and abundance should be considered in the development of assessment methodologies so that they are compliant with the WFD recommendations (WFD, 2000/60/EC). The recommendations regarding macroalgae state that the taxonomic composition should correspond to undisturbed conditions (where all sensitive taxa should be present) and that there should be no detectable changes in macroalgae abundances due to anthropogenic activities. Instead of creating a new assessment index or method, Borja and Dauer (2008) recommended that assessment schemes should integrate well-known metrics, which should create more confidence and yield advantages when interpreting the results.

In this paper, the Marine Macroalgae Assessment Tool (MarMAT) is presented. The MarMAT was developed to assess the ecological status of a system based on the macroalgae found within a system's intertidal rocky shores. The MarMAT combines the philosophy of assessment tools that have already been tested and are being used around EU countries, such as the RSL (Wells et al., 2007), the CFR (Juanes et al., 2008), the EEI (Orfanidis et al., 2001, 2003), and the opportunistic macroalgae assessment method (Scanlan et al., 2007; Patrício et al., 2007). The first version of the MarMAT (the P-MarMAT) was intercalibrated with the CFR (Spanish tool) during the first phase of the European Intercalibration (IC) Exercise. The P-MarMAT achieved an excellent agreement value with the Spanish tool (0.89 from a Kappa analysis) (E.C., 2008; Carletii and Heiskanen, 2009). Gaspar et al. (2012) defined the ecological reference conditions and the quality classes for several indicators of macroalgae. Following this, the MarMAT was updated, both for the metrics and for the reference conditions. The MarMAT fulfils the WFD requirements for abundance and taxonomic composition because the selected metrics are based on macroalgal attributes, such as species composition; diversity among Chlorophyta, Rhodophyta, and Heterokontophyta (Phaeophyceae); and the biomass or coverage of some taxa that allow these communities to be characterised.

Although species composition is expected to vary successively over time (e.g., days, seasons, and years) as a result of environmental changes (e.g., natural or anthropogenic disturbances) or of natural differences between sites (Addessi, 1994; Keough and Quinn, 1998; Lindberg et al., 1998; Panayotidis et al., 2004; Arévalo et al., 2007; Krause-Jensen et al., 2007, 2008; Gaspar et al., 2012) species richness remains approximately constant in the absence of environmental modifications (Wells and Wilkinson, 2002, 2003; Gaspar et al., 2012). Variations in composition are mainly due to changes in transient taxa, and species richness in intertidal rocky shore communities remains approximately constant under constant environmental conditions (Wells and Wilkinson, 2002, 2003).

Under environmental degradation (i.e., water transparency, nutrient enrichment) macroalgal communities decrease in diversity (e.g., elimination of sensitive species) and increase in biomass of opportunist species due to environmental stimulation (Orfanidis et al., 2003; Arévalo et al., 2007; Krause-Jensen et al., 2007; Scanlan et al., 2007; Patrício et al., 2007; Gaspar et al., 2012). When exposed to nutrient-enriched waters, opportunist species can dominate the community at the expense of larger and perennial algae (Schramm, 1999; Orfanidis et al., 2003; Krause-Jensen et al., 2007, 2008). During such occasions, a shift in marine ecosystems' structure and function from a pristine to a degraded state may occur; the replacement of late succession seaweeds by opportunistic species is a reliable signal of increasing eutrophication (Orfanidis et al., 2001, 2003). Orfanidis et al. (2001, 2011) considered two Ecological Status Groups (ESGs): ESG I (late succession or perennial to annual taxa) and ESG II (opportunist or annual taxa). ESG I includes seaweeds with thick or calcareous talus, low growth rates and long life cycles, whereas ESG II includes sheet-like thin simple tissue and filamentous species with high growth rates and short life cycles (usually annual) (Orfanidis et al., 2001, 2003). The ratio between these two groups of species has been used as a measure of environmental degradation; lower values correspond to deteriorating ecological conditions (Orfanidis et al., 2001, 2011).

Another factor influencing species richness is the morphology of rocky shores. Wells et al. (2007) demonstrated statistically that substrata can influence variations in species richness observed among shores. Rock ridges, outcrops and platforms have a significantly higher number of species than shores consisting predominantly of boulders, pebbles and vertical rock. The shore description, with different scores attributed to different shores' morphology, constitutes an important factor to include (as a species richness correcting factor) in assessment methodologies.

The present study aims to (a) select a group of relevant metrics to include in an assessment tool (i.e., the MarMAT method); (b) test the tool's response against different anthropogenic pressure levels; (c) analyse its performance; and (d) compare its performance to the performance of other assessment tools currently in use by EU countries.

2. Materials and methods

2.1. Study site

The study area is located along the western coast of Portugal (Fig. 1). It is located inside the EU North-East Atlantic (NEA) region, typology NEA 1 (WFD, 2000/60/EC) which is equivalent to the Portuguese type A5 (Bettencourt et al., 2004). This region of the coast is an open and exposed euhaline and mesotidal (1–3 m amplitude) coastal area that is frequently turbid and nutrient-enriched due to coastal upwelling (Ambar and Dias, 2008).

During the summer, the Canary Current, which has a strong southward flow (12 $\mathrm{cm\,s^{-1}}$) originating from the north, and the Azores Current, which enters the region from the south and has a west-to-east circulation, affect the Portuguese coast. During the winter, the Azores Current has twice the velocity it has in the summer, and there is little circulation of seawater in the region. The circulation of seawater along the Iberian Coast flows predominantly south to north with a velocity of approximately 1.6 $\mathrm{cm\,s^{-1}}$ (Ambar and Dias, 2008).

Sampling was conducted at nine intertidal rocky shore sites located along the study area: the Vila Praia de Âncora (VPA), Montedor (M), Viana do Castelo (VC), Cabedelo (Ca), Lavadores (La), Aguda (Ag), Buarcos Bay (BB), São Martinho do Porto (SMP) and Peniche (P) shores (Fig. 1). These sites experience different levels of anthropogenic pressure; eight of these sites (Table 1) were selected to test

Table 1
Sampling sites names, codes, and dates. Information on which sites were assessed for anthropogenic pressures and the location from which the pressure information is thought to initiate.

Sampling sites	Site code	Sampling date	Assessed against pressure	Pressures related to location
Vila Praia Ancora	VPA-3	July 2010		
Montedor	M-3	July 2010	Yes	Caminha
Viana Castelo	VC-5	August 2007	Yes	Viana do Castelo
Viana Castelo	VC-6	July 2010		
Cabedelo	Ca-2	July 2007		
Cabedelo	Ca-3	September 2007	Yes	Oporto
Lavadores	La-2	July 2007		
Lavadores	La-3	September 2007	Yes	Vila Nova de Gaia
Aguda	Ag-2	July 2007		
Aguda	Ag-3	September 2007	Yes	Espinho
Buarcos Bay	BB-9	October 2007		
Buarcos Bay	BB-10	June 2008		
Buarcos Bay	BB-11	June 2009		
Buarcos Bay	BB-12	September 2009	Yes	Figueira da Foz
Buarcos Bay	BB-13	July 2010		
Sao Martinho do Porto	SMP-2	August 2009	Yes	Alcobaca
Peniche	P-3	September 2009	Yes	Peniche

the response of the MarMAT to different levels of environmental stress (tool validation).

2.2. Biological data

Sampling was performed during low tide on the intertidal rocky substrates, primarily during the summer and spring (Table 1). All data resulted from non-destructive quantitative assessments and were restricted to a shore sample collected from a single low tide event. A 10–15 m band located perpendicular to the water line was chosen to represent the macroalgal populations at each site. Within that sampling band, all macroalgal taxa observed were recorded (to the species level or closest relative) so that the taxonomic composition could be estimated. A transect was defined within the banded area and perpendicularly to the water line to simultaneously record the site's macroalgal abundance (coverage of opportunistic taxa). Seven samples were collected at each site; each sample was collected at a different intertidal depth levels (one representative level for each main intertidal zone: lower-, mid- and upper-littoral; one intermediate level above and one intermediate level below those zones). This was accomplished by photographing a 0.2 m × 0.2 m wire quadrat (sub-divided into 16 sub-quadrats) positioned along the transect and located over the rocky substratum covered by macroalgae. A sample was considered to be three replicates (three photographed quadrats) placed perpendicular to the transect line. Twenty-one replicates per transect were analysed.

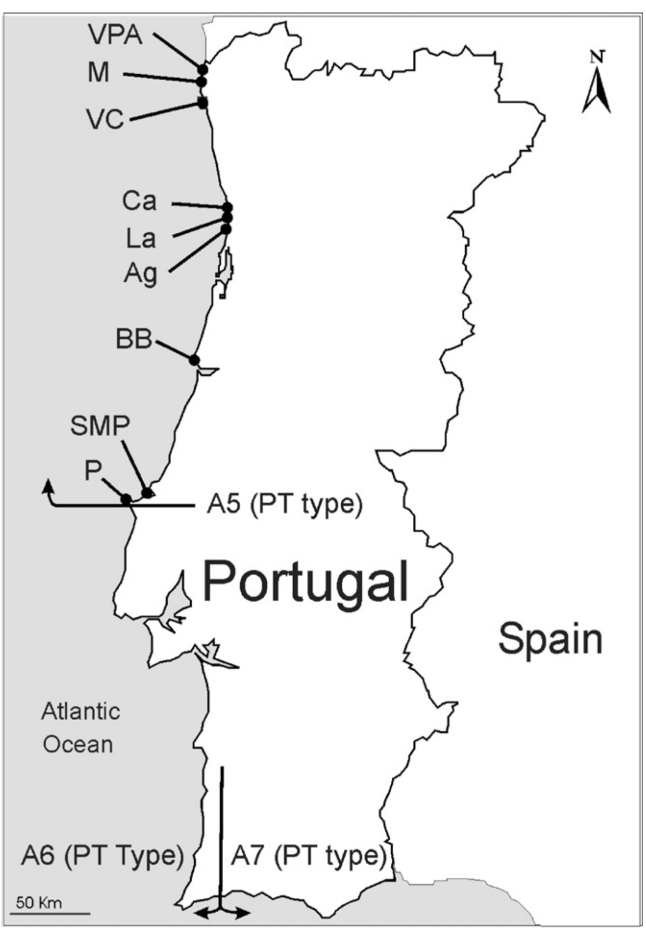

Fig. 1. Sampling sites of the A5 coastal waters, Portuguese (PT) typology (EU NEA 1) included in the study: Vila Praia de Âncora (VPA), Montedor (Mo), Viana do Castelo (VC), Cabedelo (Ca), Lavadores (La) and Aguda (Ag), Buarcos Bay (BB), São Martinho do Porto (SMP) and Peniche (P).

2.3. Marine Macroalgae Assessment Tool (MarMAT)

The MarMAT includes seven different metrics: species richness, proportion of Chlorophyta, number of Rhodophyta, number of opportunists/ESG I (ratio), proportion of opportunists, shore description, and coverage of opportunists.

A group of metrics that can adequately integrate into a WFD-compliant assessment tool were selected based on results outlined by Gaspar et al. (2012). Similar to other assessment tools (e.g., the RSL and the CFR), the MarMAT is based on a Reduced Taxa List (RTL) (Table 2) adapted to the variety of shore typology. The RTL considered in this study was developed by Gaspar et al. (2012) for the same study area.

Species richness, proportion of Chlorophyta, number of Rhodophyta, proportion of opportunists, and the ratio of the number of opportunists/ESG I were calculated based on taxa in the RTL. The coverage of opportunists was estimated from the photographed quadrats, having in mind the species considered as opportunists in the RTL.

Considering only the taxa in the RTL, the metrics were defined as follows:

- *Species richness* was the number of taxa recorded in the community. Due to the importance in distinguishing between the quality of shore sites, this metric was weighted twice (factor of 2).

Table 2
Reduced Taxa List (RTL) for the A5 Portuguese Coastal Water (CW) typology (NEA 1 for Portuguese northern coast). ESG = Ecological Status Groups.

Reduced Taxa List (CW A5 PT type)	ESG	Opportunistic
Chlorophyta		
Bryopsis spp.	II	Yes
Other Filamentous Chlorophyta (1)	II	Yes
Cladophora spp.	II	Yes
Codium spp.	II	
Ulva spp. ('Sheet-type')/Ulvaria *obscura*/*Prasiola stipitata* (2)	II	Yes
Ulva spp. ('Tubular-type')/*Blidingia* spp. (3)	II	Yes
Phaeophyceae (Heterokontophyta)		
Bifurcaria bifurcata	I	
Cladostephus spongiosus	I	
Colpomenia spp./*Leathesia marina*	II	
Cystoseira spp.	I	
Desmarestia ligulata	II	
Dictyopteris polypodioides	II	
Dictyota spp.	II	
Filamentous Phaeophyceae (4)	II	Yes
Fucus spp.	I	
Halopteris filicina/*H. scoparia*	II	
Himanthalia elongata		
Laminaria spp.		
Pelvetia canaliculata		
Ralfsia verrucosa		
Saccorhiza polyschides		
Rhodophyta		
Acrosorium ciliolatum/*Callophyllis laciniata*/*Cryptopleura ramosa*	II	
Ahnfeltia plicata	I	
Ahnfeltiopsis spp./*Gymnogongrus* spp.	II	
Apoglossum ruscifolium/*Hypoglossum hypoglossoides*	II	
Asparagopsis armata/*Falkenbergia rufolanosa*	II	
Bornetia spp./*Griffithsia* spp.	II	
Calliblepharis spp.	I	
Catenella caespitosa/*Caulacanthus ustulatus*	II	
Champiaceae (5)	II	
Chondracanthus acicularis	II	
Chondracanthus teedei	II	
Chondria spp.	II	
Chondrus crispus	I	
Calcareous encrusters (6)	I	
Calcareous erect (7)	I	
Dilsea carnosa/*Schizymenia dubyi*	II	
Gelidiales (8)	I	
Gigartina pistillata	II	
Gracilaria spp.	II	
Grateloupia filicina	II	
Halurus equisetifolius	II	
Hildenbrandia spp.	I	
Laurencia spp./*Osmundea* spp.	II	
Mastocarpus stellatus/*Petrocelis cruenta*	I	
Nitophyllum punctatum	II	
Other Filamentous Rhodophyta (9)	II	Yes
Phyllophora spp./Rhodymenia pseudopalmata	II	
Palmaria palmata	I	
Peyssonnelia spp.	I	
Plocamium cartilagineum/*Sphaerococcus coronopifolius*	I	
Porphyra spp.	II	Yes
Pterosiphonia complanata	II	
Scinaia furcellata	I	

(1) *Chaetomorpha, Pseudendoclonium, Rhizoclonium*, Ulothricales. (2) *Ulva* spp. 'Sheet-type' in opposition to (3) 'Tubular-type' in the sense of '*ex-Enteromorpha* spp.' (4) Ectocarpales/*Sphacelaria* spp. (5) *Champia, Chylocladia, Gastroclonium, Lomentaria*. (6) *Lithophyllum, Melobesia, Mesophyllum, Phymatolithon*. (7) *Amphiroa, Corallina, Jania*. (8) *Gelidium, Pterocladiella*. (9) *Acrochaetium, Aglaothamnion, Antithamnion, Bangia, Boergeseniella, Brongniartella, Colaconema, Callithamnion, Ceramium, Compsothamnion, Dasya, Erythrotrichiaceae, Herposiphonia, Heterosiphonia, Janczewskia, Leptosiphonia, Lophosiphonia, Ophidocladus, Pleonosporium, Plumaria, Polysiphonia, Pterosiphonia* (except *P. complanata*), *Pterothamnion, Ptilothamnion, Rhodothamniella, Streblocladia, Vertebrata*.

- The *proportion of Chlorophyta*, was the number of species from this Class divided by the total number of species recorded in the community.
- The *number of Rhodophyta* was the number of species from this Class recorded in the community.
- The *number of opportunists/ESG I* was the ratio calculated between the number of opportunists and the number of taxa belonging to ESG I (late successional or perennial taxa).
- The *proportion of opportunists*, was given by the number of species classified as such, divided by the total number of species recorded in the community.

In degraded habitats, an increase in the number of opportunist species and an extension of their coverage area was expected to occur (see Gaspar et al., 2012). In fact, the competitive advantage of opportunists tends to lead to the elimination of sensitive species

Table 3
Field sampling form for the shore descriptions.

General information						
Shore name		Date				
Water body		Tidal height				
Latitude/Longitude		Time of low tide				
Shore descriptions						
Presence of turbidity	Yes = 0	Sand scour	Yes = 0	No = 2		
(known to be non-anthropogenic)	No = 2	Chalk shore	Yes = 0	No = 2		
Dominant shore type			Subhabitats			
Rock ridges/outcrops/platforms		4	Wide shallow rock pools			
Irregular rock		3	(>3 m wide and <50 cm deep)	4		
Boulders large, medium and small		3	Large rockpools (>6 m long)	4		
Steep/vertical rock		2	Deep rockpools (50% >100 cm deep)	4		
Non-specific hard substrate		2	Basic rockpools	3		
Pebbles/stones/smallrocks		1	Large crevices	3		
Shingle/gravel		0	Large overhangs and vertical rock	2		
			Others habitats (please specify)	2		
Dominant biota			Caves	1		
Ascophyllum			None	0		
Fucoid						
Rhodophyta mosaics		Total number of sub-habitats				
Chlorophyta		>4	3	2	1	0
Mussels						
Barnacles		General comments				
Limpets						
Periwinkles						
Sum of categories' scores		N/A	15–18	12–14	8–11	1–7
Shore description equivalent score		0	1	2	3	4

Adapted from Wells et al. (2007).

and to an increase in biomass and coverage area for r-selected species. The *coverage of opportunists* (CO), expressed as a percentage, is given by the area covered by these taxa (only opportunistic taxa included in the RTL) in relation to the whole area covered by the macroalgae (all species corresponded to 100% coverage). Each photographed replicate was analysed using the following formula (Eq. (1)):

$$\mathrm{CO}(\%) = \frac{\mathrm{QCO} \times 100}{16 - \mathrm{EQ}} \quad (1)$$

where QCO is the number of sub-quadrats with opportunistic macroalgae and EQ is the number of empty sub-quadrats. A resolution of ¼ of a sub-quadrat was used for the calculations.

The CO was calculated as the arithmetic average of all of the replicates from a site. Because the CO is the only metric in the MarMAT that accounts for abundance, one of the parameters required by the WFD, it was double weighted (factor of 2).

The objective of including a *shore description* metric was to make shores with different substrata comparable and, consequently, make different environmental conditions for macroalgae growth comparable. Shore descriptions serve as a correction factor for species richness scores, which is a metric included in the MarMAT. For this purpose, Wells et al. (2007) proposed that a field sampling form should be used to record basic shore descriptions during sampling visits (Table 3). The numbers on the sampling sheet attached to each of the shore/habitat categories are based on how much they contribute to the overall species richness (Wells et al., 2007). Information on the dominant biota, although it does not contribute to the overall scoring system, may be useful in subsequent years to explain ecological changes, if they occur (Wells et al., 2007). Scores from each of the categories were added together, and depending on their range, an equivalent score (Table 3) was used to calculate the final classification. Only the highest score was used to estimate the sum of categories' scores for categories with more than one description recorded (e.g., shore type and habitat type) (Wells et al., 2007).

The value of each of these metrics varied from 0 to 4; this range was divided into five intervals that corresponded to the five quality classes, in accordance with the Normative Definitions (annex V in WFD) (WFD, 2000/60/EC): bad – 0, poor – 1, moderate – 2, good – 3, and high – 4 (Table 4).

The scores of the different metrics were integrated to provide an overall classification of the shore. For example, if 15 taxa were found on a given shore, the shore received a score of 2 for species richness (moderate); if the proportion of Chlorophyta on a given shore was 0.15, the shore received a score of 3 (good) (Table 3). The sum of the scores obtained for the different individual metrics was integrated in the 'sum of scores' (0–36).

The EQR (Eq. (2)) converts the 'sum of scores' values to a scale from 0 to 1, in accordance with the definition provided in the WFD (WFD, 2000/60/EC).

$$\mathrm{EQR} = \frac{\text{Sum of Scores}}{36} \quad (2)$$

EQR values close to 1 correspond to high quality ecological status, while EQR values close to 0 correspond to low quality ecological status. The 0–1 EQR interval is subsequently translated into the EQS classes (bad, poor, moderate, good and high) using the boundaries provided in Table 4.

2.4. Assessment of anthropogenic pressures

Three indicators were considered to be proxies of anthropogenic pressures that may influence the sampling sites: (a) urban land use (represented by the number of inhabitants); (b) industrial land use; and (c) agricultural, forest and fishing areas (*sensu* the Land Uses Simplified Index (LUSI); Royo et al., 2009). This method of assessing the anthropogenic pressures that affect coastal areas was based on pressures identified from land, which may be related to impacts (e.g., macroalgal communities degradation) observed in these zones.

Pressures were calculated from information available from the National Institute of Statistics (http://www.ine.pt) for the study period and were scored from 1 to 4 following the criteria shown in Table 5. Eight sites were analysed; the locations (cities) considered to have a significant influence on the anthropogenic pressure level for each site are shown in Table 1.

Table 4
Boundaries for the selected metrics (Gaspar et al., 2012), sum of scores and EQR. Translation of the achieved EQR in the EQS (bad, poor, moderate, good or high) when assessing the ecological quality of rocky shores.

Metrics	Bad	Poor	Moderate	Good	High
Species richness (a)	0 – 6	7 – 13	14 – 20	21 – 27	28 – 54
Proportion of Cholophyta	0.32 – 1	0.27 – 0.31	0.21 – 0.26	0.15 – 0.20	0 – 0.14
Number of Rhodophyta	0 – 3	4 – 8	9 – 12	13 – 17	18 – 33
Number of opportunists / ESG I	≥1.23	1.01 – 1.22	0.80 – 1.00	0.58 – 0.79	<0.58
Proportion of opportunists	0.59 – 1	0.47 – 0.58	0.35 – 0.46	0.23 – 0.34	0 – 0.22
Coverage of opportunists (%) (a)	72 – 100	59 – 71	46 – 58	33 – 45	0 – 32
Shore Description	–	15 – 18	12 – 14	8 – 11	1 – 7
Corresponding score to metrics class	0	1	2	3	4
Sum of scores	0 – 7	8 – 14	15 – 21	22 – 28	29 – 36
EQR	0 – 0.20	0.21 – 0.40	0.41 – 0.60	0.61 – 0.80	0.81 – 1
EQS	Bad	Poor	Moderate	Good	High

(a) Factor of 2, counts twice in the metrics sum of scores calculation.

Table 5
Criteria used to assess anthropogenic pressures. Indicators of anthropogenic pressure and years considered for the assessment.

	Scores			
	1	2	3	4
No. inhabitants × 1000 (2008)	<350	<700	<1050	<1400
Industrial land use (ha) (2008)	<1250	<2500	<3750	<5000
Agriculture/Forest/Fishing surface area (ha) (1999)	<4500	<9000	<13,500	<18,000

The total pressures were compared with the EQR from sampling sites to validate the response of the MarMAT against anthropogenic pressures. The correlation between both data series (MarMAT EQR and anthropogenic total pressures) was tested through the Pearson product moment correlation coefficient, with StatSoft Inc. (2004) STATISTICA (data analysis software system), version 7.

2.5. MarMAT performance

Four sets of results were calculated to analyse the performance of the MarMAT:

- the MarMAT presented in this study;
- the MarMAT without the CO metric;
- the RSL (Wells et al., 2007) with metric boundaries of the MarMAT;
- the RSL (Wells et al., 2007) with RSL original metric boundaries.

For both calculations of the RSL, the species list used was the RTL due to its local suitability.

These results enabled the following to be assessed: (a) the ability of the MarMAT to report values for all of the five quality classes; (b) the ability of the MarMAT to confirm the importance of using the abundance parameter in the CW quality assessment; and (c) the importance of considering reference conditions adapted to the eco-region under assessment.

The correlation between each pair of results was tested through the Pearson product moment correlation coefficient, with StatSoft Inc. (2004) STATISTICA (data analysis software system), version 7.

3. Results

Table 6 summarises the EQR's values resulting from the application of the MarMAT, the MarMAT without CO, RSL with the MarMAT boundaries, and RSL with the RSL original boundaries. The EQS is expressed by the corresponding colour (see Table 4).

The MarMAT results ranged from 0.19 (bad) to 0.94 (high) (Fig. 4). All quality classes except for 'moderate' were obtained by the MarMAT. The MarMAT without CO and the RSL with the MarMAT boundaries had EQS scores ranging from poor to high. The RSL with the RSL original boundaries did not capture results lower than the moderate quality class. Although all the applications were correlated to one other, the correlation between the MarMAT and the MarMAT without CO had the highest value ($p < 0.001$; $n = 17$; $r^2 = 0.92$), and the correlation between the RSL with the MarMAT boundaries and the RSL with the RSL original boundaries had the lowest value ($p < 0.001$; $n = 17$; $r^2 = 0.82$).

Values were calculated to assess the anthropogenic pressures affecting the sampling sites (values ranged from 3 to 12) (Fig. 2). Higher anthropogenic pressure values were obtained for Cabedelo (Ca), a site influenced by the city of Oporto (see details in Gaspar et al., 2012). Somewhat lower anthropogenic pressure values were obtained for Lavadores (La) and for São Martinho do Porto (values ranged from 5 to 6). Low pressures values were obtained for three sites, including Montedor (M), Aguda (Ag) and Peniche (P) (value of 3).

The response of the ecological assessment performed by the MarMAT against the anthropogenic pressure level quantified for sampling sites is shown in Fig. 3. The total anthropogenic pressure

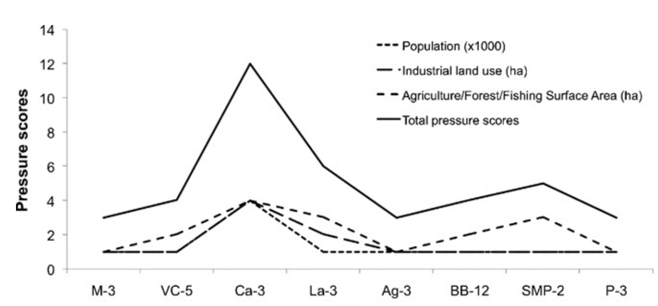

Fig. 2. Quantification of anthropogenic pressures observed along the study area. Number of inhabitants; industrial land use (ha); agriculture/forest/fishing surface area (ha). Study sites: Montedor (M); Viana do Castelo (VC); Cabedelo (Ca); Lavadores (La); Aguda (Ag); Buarcos Bay (BB); São Martinho do Porto (SMP); Peniche (P).

Table 6
Assessment results for the MarMAT, the MarMAT without coverage of opportunists, the RSL with the MarMAT boundaries, and the RSL with the RSL original boundaries. Values for metrics (a – used in the MarMAT; b – used in the RSL) and EQR and colours for EQS are included (see Table 4).

	VPA-3	M-3	VC-5	VC-6	Ca-2	Ca-3	La-2	La-3	Ag-2	Ag-3	BB-9	BB-10	BB-11	BB-12	BB-13	SMP-2	P-3
Species richness (a, b)	34	34	32	37	11	8	21	20	37	31	31	31	33	34	37	29	36
No. of Chlorophyta	5	4	4	4	3	2	4	3	6	3	3	3	4	4	4	4	6
No. of Rhodophyta (a)	20	20	19	21	6	6	13	13	22	19	21	21	22	23	26	16	20
No. of opportunists	6	6	5	4	6	4	6	5	8	5	4	5	5	5	6	4	7
ESG1	9	12	13	15	4	3	7	7	12	10	10	9	10	9	11	13	12
ESG2	25	22	19	22	7	5	14	13	25	21	21	22	23	25	26	16	24
Proportion of Cholophyta (a, b)	0.15	0.12	0.13	0.11	0.27	0.25	0.19	0.15	0.16	0.10	0.10	0.10	0.12	0.12	0.11	0.14	0.17
Proportion of Rhodophyta (b)	0.59	0.59	0.59	0.57	0.55	0.75	0.62	0.65	0.59	0.61	0.68	0.68	0.67	0.68	0.70	0.55	0.56
Proportion of opportunists (a, b)	0.18	0.18	0.16	0.11	0.55	0.50	0.29	0.25	0.22	0.16	0.13	0.16	0.15	0.15	0.16	0.14	0.19
ESG Ratio (b)	0.36	0.55	0.68	0.68	0.57	0.60	0.50	0.54	0.48	0.48	0.48	0.41	0.43	0.36	0.42	0.81	0.50
No. of opportunists / ESG I (a)	0.67	0.50	0.38	0.27	1.50	1.33	0.86	0.71	0.67	0.50	0.40	0.56	0.50	0.56	0.55	0.31	0.58
Coverage of opportunists (%) (a)	73	38	29	57	98	78	33	48	50	45	37	63	65	48	36	32	26
Shore description (a, b)	2	2	2	2	2	2	3	3	2	2	2	2	2	2	2	2	2
MarMAT	0.69	0.89	0.94	0.83	0.19	0.22	0.72	0.64	0.78	0.89	0.89	0.78	0.78	0.83	0.89	0.92	0.89
MarMAT without Coverage of Opportunists	0.89	0.93	0.93	0.93	0.25	0.29	0.71	0.68	0.86	0.93	0.93	0.93	0.93	0.93	0.93	0.89	0.86
RSL with MarMAT boundaries	0.63	0.75	0.83	0.75	0.33	0.58	0.71	0.75	0.71	0.79	0.83	0.79	0.79	0.75	0.79	0.75	0.63
RSL with RSL original boundaries	0.75	0.79	0.88	0.88	0.54	0.58	0.75	0.75	0.75	0.75	0.79	0.75	0.83	0.79	0.79	0.83	0.79

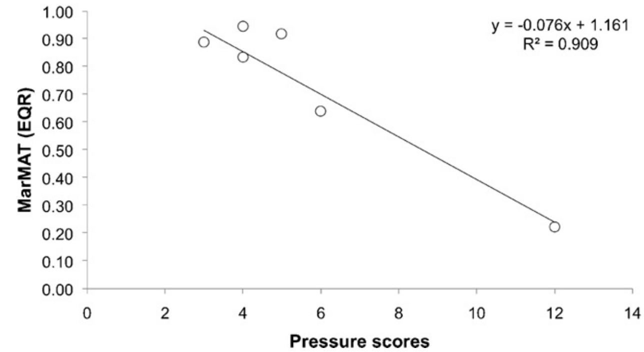

Fig. 3. . Plot of EQRs reported by the MarMAT versus the total anthropogenic pressures for sites M-3, VC-5, Ca-3 La-3, Ag-3, BB-12, SMP-2, and P-3 (see Table 1).

and the EQR values had a significant inverse correlation ($p < 0.001$; $n = 8$; $r^2 = 0.91$).

4. Discussion

The MarMAT, which is firmly based on scientific knowledge, is easy to use and can rapidly provide assessment results. Most of the metrics included in the MarMAT were previously considered in tools developed in other EU MSs for the same purpose, such as the RSL (Wells et al., 2007) in the UK, the CFR (Juanes et al., 2008) and the CARLIT (Ballesteros et al., 2007) in Spain, and the EEI (Orfanidis et al., 2003) in Greece. Nevertheless, in addition to using a list of species that are easy to identify in the field and a set of other metrics estimated from taxa belonging to this list (e.g., the proportion of Chlorophyta and the proportion of opportunists), the results from the MarMAT tests are improved by including a coverage value, as first mentioned in the CFR (Juanes et al., 2008). This last metric was included to fulfil the abundance parameter required by the WFD, and a factor of 2 was applied to emphasise its importance. This improvement was considered advisable according to Guinda et al. (2008), who compared the performances of the CFR (which includes a coverage metric) and the RSL (which does not include a coverage metric) and concluded that the CFR responded more accurately to the analysed pollution gradients.

The metrics that responded best to the detection of environmental degradation were evaluated (Gaspar et al., 2012). These metrics were not very different from the ones initially proposed by Wells et al. (2007) for the RSL method, but due to progress made in the scientific field concerning the classification of macroalgal taxa (ESG and opportunistic species) (Orfanidis et al., 2011), some metrics were replaced. The number of Rhodophyta showed a stronger

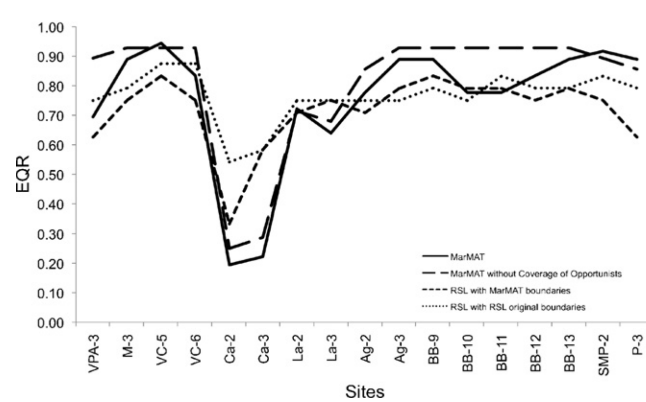

Fig. 4. Assessment results. The EQRs reported for the sample sites by the MarMAT, the MarMAT without coverage of opportunists, the RSL with the MarMAT boundaries, and the RSL with the RSL original boundaries.

correlation with anthropogenic pressure values than its proportion. Therefore, the former is now included in the MarMAT. After the ESG's reclassification (made by Orfanidis et al. (2011) for macroalgal taxa) the number of opportunists/ESG I metric was more efficient in detecting degradation of macroalgal communities due to the presence of anthropogenic pressures than the ESG I/ESG II metric. Therefore, the former replaced the latter in MarMAT.

The use of a large dataset (including historical and monitoring data) allowed Gaspar et al. (2012) to improve the robustness of the RTL for the coastal study area (A5 Portuguese CW type) as well as improve the reference conditions for several macroalgae metrics. For this reason, the RTL and the reference conditions determined by Gaspar et al. (2012) were used in the present study. To verify that the MarMAT will perform well when different CWs are assessed, the RTL must be correctly adapted, and it must include the most representative taxa of each CW typology.

In the present study, the pollution gradient (anthropogenic pressure) affecting the sampling sites were assessed through the quantification of anthropogenic pressures known to disturb coastal areas. These are considered to be proxies of disturbances impacting coastal communities; this concept is based on anthropogenic land use (Table 5). Clearly identifying pressures affecting a site is essential when evaluating a specific influence or effect. Because the differences observed in macroalgal communities are attributable to various environmental factors (Ballesteros et al., 2007), such as nutrients, temperature or currents, it is clear that the human presence in coastal areas may influence the variation of those parameters. A quantification of anthropogenic pressures that considers the magnitude of human presence around coastal sites, such as the concept outlined in the LUSI (Royo et al., 2009), can be used when it is difficult to directly quantify anthropogenic pressures. The results reported in this study (Fig. 2) support this notion and suggest its applicability to similar situations.

In general, the MarMAT provided EQS classifications that met expectations. As suggested by the WFD, the data used in this study consisted of primarily summer samples so that a higher homogeneity of sampling conditions was guaranteed and both the seasonal episodic explosion of transient species, which occurs in April–May, and poor weather conditions, which make sampling more difficult during the autumn and winter, were avoided. Such conventions may need to be adapted in other geographical areas (Ballesteros et al., 2007).

The MarMAT successfully captured the total anthropogenic pressure calculated for the sites (Fig. 3). This validates the MarMAT methodology in terms of the requirements listed in the WFD for the behaviour of assessment tools. Another important feature that assessment tools must incorporate is the ability to report the five quality classes (bad to high). The MarMAT results indicated that the Cabedelo site (Ca-2) had a bad EQS (EQR = 0.19) and that the Montedor, Viana do Castelo, and Aguda sites had high EQS (e.g., EQR = 0.94) (Table 6, Fig. 4). In addition, a classification of high EQS was obtained whenever the total anthropogenic pressure was low along the length of the study area. This indicates that the list of taxa, the RTL, is balanced and does not restrict the outputs of the tool.

Concerning the comparison made between the MarMAT and the RSL (on the four variations presented) it is important to highlight that the MarMAT was the most efficient at discriminating the ecological status of the several sites (Fig. 4). MarMAT was the only assessment tool that successfully obtained the complete range of quality classes. The MarMAT without CO was also efficient, but it did not obtain the bad EQS class (Ca-2) and did not lower the classification from high to good in BB-10, BB-11 and BB-12. In general, the classification provided by this version was higher than the classification provided by the MarMAT. Apparently, the inclusion of the CO metric resulted in a higher accuracy of the assessment. Both versions of the RSL performed worse than the MarMAT. The RSL versions obtained a narrower range of quality classes and failed to obtain the lowest classes. As a general trend, the RSL with RSL original boundaries performed the worst and had higher EQR values. This suggests that the boundaries should be adapted to the geographical differences of a study site. The version that used boundaries adapted to the study area obtained lower EQR values for Ca-2 than the version using boundaries not adapted to local conditions.

Boundaries adopted here were equidistant (0.20) but they may be improved or adjusted through comparison and intercalibration of the MarMAT with other methodologies (e.g., the RSL and the CFR). This procedure will ensure compliance regarding EQS assessments in contiguous coastal areas and the WFD.

5. Conclusions

The results of the present study illustrate that macroalgae can efficiently integrate the effects of different environmental conditions and are therefore good ecological indicators of water quality.

The Marine Macroalgae Assessment Tool (MarMAT) proposed in the present study is compliant with the WFD recommendations regarding the need to evaluate parameters such as abundance and taxonomic composition. Moreover, the macroalgae Reduced Taxa List (RTL) was robust in representing the natural variability of macroalgal taxa in the northern Portuguese coastal waters (CW).

The response of the MarMAT against the anthropogenic pressures was in accordance with expectations, consistently providing the worst EQS classifications at sites reporting higher total anthropogenic pressure values. In addition, inclusion of the geographical adaptation of the reference conditions and the boundaries is important to improve the reporting accuracy of assessment methods. The MarMAT constitutes an efficient assessment tool for macroalgal communities.

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Development of a tool for assessing the ecological quality status of intertidal coastal rocky assemblages, within Atlantic Iberian coasts

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ABSTRACT

The aim of this paper is to develop a new methodology for assessing the quality of coastal waters along the Atlantic Iberian coasts, based upon Basque coast rocky intertidal assemblages, compliant with the European Water Framework Directive (WFD, 2000/60/EC). Biological data collected over a 20-year period, during the gradual introduction of a sewerage plan, are compared to several reference stations in order to differentiate various degrees of community alteration. A quality index (RICQI: Rocky Intertidal Community Quality Index) is drawn up, on the basis of: indicator species abundance; morphologically complex algae cover; species richness; and faunal cover (herbivore and suspensivore cover, proportion of fauna with respect to the whole assemblage). An independent dataset collected in Plentzia Bay (Basque coast, N. Spain), before and after the set-up of a wastewater treatment plant, is used in order to validate RICQI. A conceptual model based on our results is proposed, which describes successional stages of assemblages along a gradient of increasing environmental disturbance and associated values of the metrics included in the index. The performance of this new approach is compared with that of the quality of rocky bottoms index, used presently as the official method for assessing the ecological status of rocky assemblages in the Atlantic coastal waters of Spain. Both indices respond to changes in community structure, associated with pollution removal. However, the RICQI index shows a more accurate response, identifying different degrees of disturbance.

1. Introduction

The European Water Framework Directive (WFD, 2000/60/EC) establishes a framework to prevent deterioration and protect aquatic ecosystems. The main objective of this Directive is to achieve a 'good ecological status', for all waters, by 2015. The Biological Quality Elements (BQEs) determined by the WFD, for assessing the ecological status in coastal waters, include phytoplankton, macroalgae, angiosperms and macroinvertebrates. The application of the WFD has encouraged scientists to work on the design of different methodologies, for assessing ecological status. Regarding coastal waters, applied ecologists have invested considerable amount of effort and, now, there is an increasing number of methods in use for assessing each BQE (European Commission, 2008; Borja et al., 2009). Such methods must be based upon a comparison between monitored data and reference conditions (unaffected by human pressures), calculating an Ecological Quality Ratio (EQR), ranging from 0 (worst status) to 1 (best status), capable of classifying the water bodies into one of the five status classes: Bad, Poor, Moderate, Good and High (see Borja, 2005).

However, methods or indices for assessing macroalgae are not as well developed as assessment methods for the other BQEs (Borja et al., 2012). Most of the macroalgae assessment methods include some measurement of richness (even in terms of presence/absence) and abundance (generally, as the percentage of cover, but also as biomass). Several methods utilise the ecological or functional groups (Orfanidis et al., 2001), or the presence of indicator species (opportunistic or sensitive) as a way of detecting disturbances. In Europe, the methods used most widely include the Ecological Evaluation Index (EEI) (Orfanidis et al., 2001, 2003) and the CAR-LIT (Ballesteros et al., 2007), in the Mediterranean, and the Reduced Species List (RSL) (Wells et al., 2007) and the Quality of Rocky Bottoms (CFR) (Juanes et al., 2008), in the Atlantic.

These methods utilise the algal component of the benthic community, which is considered to be an excellent indicator of stress and pollution (Arévalo et al., 2007; Mangialajo et al., 2007; Pinedo et al., 2007; Díez et al., 2010). However, few attempts have been made to develop an index for assessing the quality of hard substra-

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tum fauna (see Hiscock et al., 2005). Ecological knowledge of rocky invertebrate assemblages is capable of detecting environmental effects; its validity as a marine ecosystem indicator is extensively acknowledged (Clarke and Warwick, 1994; Hiscock et al., 2005; Rogers and Greenaway, 2005; Hiscock and Tyler-Walters, 2006). In spite of this observation, gathering the evidence necessary to distinguish between various degrees of community alteration, to establish an ecological status classification compliant with the WFD, is a somewhat challenging task.

The simultaneous use of both flora and fauna may be more appropriate, in determining the ecological status of hard substrata; this is due to the low number of invertebrate taxa, correlated with a disturbance gradient (Hiscock et al., 2005; Goodsell et al., 2009). The disposal of high loads of domestic and industrial wastewaters in the present study area has resulted in the partial replacement of macroalgae, by invertebrates (Saiz-Salinas and Isasi Urdangarin, 1994; Díez et al., 1999; Saiz-Salinas and Urkiaga-Alberdi, 1999; Pagola-Carte and Saiz-Salinas, 2001; Gorostiaga et al., 2004). Such a change in the community structure forms the basis of the method proposed for the assessment of the rocky intertidal habitat. Deviations from the natural system may be more evident, if the association between flora and fauna is studied at the same time (Underwood, 1996; Archambault et al., 2001; Bishop et al., 2002). For the development of the assessment method, the steps described by Borja and Dauer (2008) were followed, which included: (i) the spatio-temporal scale of the intended application; (ii) the selection of the candidate metrics; (iii) the metric combination; and (iv) the index validation, by testing it using an independent data set, different than the index development data set (calibration data set).

The aim of this contribution is to classify the rocky intertidal assemblages into the five ecological status classes determined by the WFD. To accomplish this objective, a new index is developed on the basis of biological data collected at several degraded sites during the gradual application of a sewerage scheme, together with several undisturbed sites. Its performance is compared here with the presently used method (the CFR), for the Atlantic coastal waters of Spain (European Commission, 2008).

2. Methodology

2.1. Spatio-temporal scale of application: the sampling area

The study area is located along the open coast, adjacent to the Abra of Bilbao (Nervión estuary), in the Basque Country (northern Spain) (Fig. 1). This area has been affected historically by human pressures; however, due to the gradual application of a sewerage plan for the metropolitan area of Bilbao (1984–2008), it has experienced an important recovery in ecosystem components, such as plankton, benthos, fishes and seabirds (Borja et al., 2010). The area under study has been monitored for coastal fauna and flora since 1984; thus, it offers data collected over comprehensive spatial and temporal scales (Pagola-Carte and Saiz-Salinas, 2001; Díez et al., 2009).

Intertidal fauna and flora were sampled bi-annually between 1996 and 2008, at four sites located along a pollution gradient (Arrigunaga, Azkorri, Meñakoz and Matxilando) (Fig. 1), where benthic communities have experienced changes in species composition and structure (Gorostiaga and Díez, 1996; Pagola-Carte and Saiz-Salinas, 2001; Díez et al., 2009). For some of the sites, biological information was analysed from years 1984 and 1992. All the surveys were undertaken in summer, to avoid seasonal variability within the phytobenthic assemblages. On each sampling event, 10 quadrats were sampled, representing the lower intertidal zone (tidal range: 0.5–1.3 m) lying closest to the open shore, on a flat or slightly sloped substratum. Pools, overhangs, unstable substrate, crevices and other different habitats were not considered, in order to reduce the natural variability associated with physical differences between the habitats. Sampling was undertaken through the use of visual assessment, where estimates of algal and animal cover were measured in 50 cm × 50 cm quadrats, following the scale proposed by Braun-Blanquet (1951): + (<1%), 1 (1–5%), 2 (5–25%), 3 (25–50%), 4 (50–75%) and 5 (75–100%). The mean cover of species among quadrats was calculated using the median of each range.

2.2. Reference conditions

As the WFD requires the determination of reference conditions in the absence of (or minimal) human pressure, two approaches were adopted: (i) a time-series dataset, collected from 1996 to 2009, at an undisturbed site (Kobaron, Fig. 1) which was taken as the reference site for the sampling sites lying adjacent to *Abra of Bilbao*; and (ii) additionally, four undisturbed sites (Borja et al., 2006) were sampled in 2009 along the Basque coast: San Juan de Gaztelugatxe (SJ), Berriatua (BE), Itziar (IT) and Jaizkibel (JA) (Fig. 1).

2.3. Candidate metrics

The WFD requires the use of composition and abundance (i.e. cover) of macroalgae, together with the disturbance-sensitive taxa presence associated with undisturbed conditions. Hence, the candidate metrics used to develop this index were: (i) cover of species with different degrees of tolerance to anthropogenic stress (SpBio), as a measure of the composition and abundance of disturbance-tolerant taxa; (ii) morphologically complex algae cover (MCA), as a proxy of the composition and abundance of the disturbance-sensitive taxa; (iii) species richness (R), as a measure of the composition; and (iv) community measures related to faunal cover (FC). The simultaneous use of both fauna and flora, for the determination of the ecological status of hard substrata is not considered by the WFD; but will be considered here, to understand further the response of hard bottom communities to human pressures. As such, it has been added in this index.

2.4. Environmental variables

In order to further explore the response of assemblages to pollution in the coastal area of the Abra of Bilbao, several environmental variables related to water quality were investigated. Abiotic data were available from 1996 to 2008 for one of the sites studied (Arrigunaga); for the remaining sites (Kobaron, Azkorri, Meñakoz and Matxilando), data were available from 2000 to 2008. Sampling was carried out four times each year, corresponding to spring, summer, autumn and winter environmental conditions. The environmental variables included turbidity in the bottom (TUb) and within the surface (TUs) layers, total suspended solids in the bottom (TSSb) and surface (TSSs) layers, organic matter in the bottom (OMb) and surface (OMs) layers, inorganic matter in the bottom (IMb) and surface (IMs) layers, as well as the light extinction coefficient (LEC). Water samples were collected with an Alpha Vertical Bottle (Wildco, USA) within 1 m from the bottom (to avoid bias from resuspended sediments) and from the surface and transferred to precleaned polypropylene containers, for transport to the laboratory. TSS, OM and IM were determined following the procedure of Moore (1972). Turbidity was measured directly by a turbidimeter (Hach 2100, U.S.A.), as Nephelometric Turbidity Units (NTU). Underwater PAR irradiance was measured ($\mu E\,m^{-2}\,s^{-1}$) every 0.5 m, to a depth of 7 m, using a LI-COR LI-192SA planar quantum sensor and a LICOR LI-1000 data logger. The visible light extinction coefficients were calculated from linear regressions of irradiance *vs.* depth.

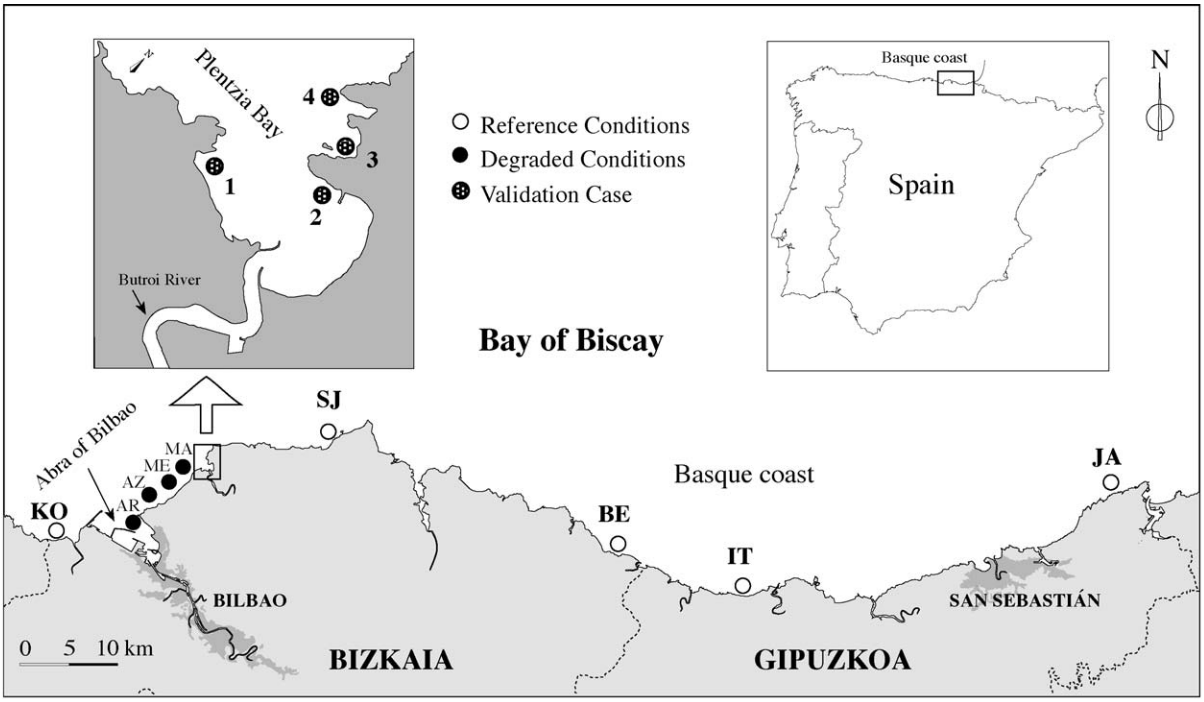

Fig. 1. Map of the sampling sites used to develop RICQI. White circles: reference conditions (KO: Kobaron; SJ: San Juan de Gaztelugatxe; BE: Berriatua; IT: Itziar; JA: Jaizkibel); black circles: degraded conditions (AR: Arrigunaga; AZ: Azkorri; ME: Meñakoz; MA: Matxilando). The insets show the sampling sites used to validate the index (dotted circles: 1. Muriola; 2. Astondo; 3. Isla Pobre; and 4. Errotatxu) and the location of the study area on the north coast of Spain.

2.5. Data analysis

The spatial and temporal patterns of data were explored by multivariate techniques, using the PRIMER V. 6. PERMANOVA *add on* software package (Clarke and Gorley, 2006, Anderson et al., 2008). As there were too many observations to establish multivariate patterns, centroids (Site × Time) were examined. Biological variables were fourth-root transformed. Non-parametric multidimensional scaling (MDS) ordinations, based upon Bray–Curtis similarity were used in order to explore patterns of assemblages. Prior to analyses a $\log(x+1)$ transformation was applied to TUb, TUs, TSSb, TSSs, OMb, OMs, IMb and IMs, in order to eliminate the skewness detected by means of draftsman plots (Anderson et al., 2008). Permutational analyses of variance (PERMANOVA) were performed, to examine differences in environmental variables. Principal Coordinates Analysis (PCO) was carried out, to visualise patterns in assemblages and the response of a whole set of environmental variables simultaneously. Only sites and years with available environmental data were included in the PCO analysis. Spearman rank correlation coefficient was used to assess the strength and direction of relationships.

Similarly, data were plotted on the basis of straightforward univariate measures of biological and environmental variables. These visual representations facilitated the interpretation of the information, by highlighting possible patterns, gradients and trends.

2.6. Metrics combination: index development

The RICQI (Rocky Intertidal Communities Quality Index) is a quantitative multimetric method, for assessing the ecological status of rocky intertidal communities on open coastal stretches of the Basque coast (probably applicable also to the Iberian Atlantic coasts), excluding extremely exposed capes, where assemblages show a different structure. Several metrics were combined in the RICQI, following the expression below.

$$\mathrm{RICQI} = \mathrm{SpBio}\,(\mathrm{ESS} + \mathrm{PC}) + \mathrm{MCA} + \mathrm{R}\,(\mathrm{Ra} + \mathrm{Rf}) + \mathrm{FC}\,(\mathrm{Pf} + \mathrm{Ch} + \mathrm{Cs})$$

Each of the terms of the expression are described below:

SpBio: Indicator species. The term SpBio consists of two components (ESS: ecological status similarity and PC: presence of *Cystoseira*). The first component (ESS) is related to the similarity between the average inventory, representing the benthic assemblages under quality evaluation (considering only the indicator species) and the five reference inventories that represent communities from bad to high ecological status (see Section 3).

As commented upon above, the WFD states that ‘all sensitive taxa should be present’ in the community, in order to attain a High ecological status. Algae of the genus *Cystoseira* are highly sensitive to anthropogenic disturbances (Benedetti-Cecchi et al., 2001; Díez et al., 2003; Thibaut et al., 2005; Arévalo et al., 2007; Pinedo et al., 2007). In the case of the Basque coast, *Cystoseira tamariscifolia* is very common in low-shore habitats of undisturbed coastal stretches (excluding extremely exposed sites); its absence could be considered as a first sign of degradation of natural communities. This indicator property is used in the RICQI, to establish differences between pristine and degraded environments by means of the PC component (which acts as a correction factor).

MCA: Morphologically complex algae. A decline in large perennial macrophytes, in response to anthropogenic disturbances, has been reported world-wide (Brown et al., 1990; Benedetti-Cecchi et al., 2001; Eriksson et al., 2002; Thibaut et al., 2005; Pinedo et al., 2007; Connell et al., 2008). By contrast, calcareous red algae are considered to be pollution-tolerant species (Bellan and Bellan-Santini, 1972; North et al., 1972; Kindig and Littler, 1980; Arévalo et al., 2007; Mangialajo et al., 2008; Díez et al., 2009) and simple forms such as filamentous and sheet-like algae proliferate in degraded environments (Fairweather, 1990; Schramm and Nienhuis, 1996; Bellgrove et al., 1997; Archambault et al., 2001; Eriksson et al., 2002; Díez et al., 2009). Three functional groups were studied, whilst developing the index: morphologically simple algae, calcareous algae, and MCA. Of those, only MCA was selected as a component of RICQI.

R: Species richness. The term R consists of two components (Ra: algal species richness and Rf: faunal species richness). It is acknowledged widely that species richness is a community measure that reflects environmental health (Bianchi and Morri, 2000; Archambault et al., 2001; Soltan et al., 2001; Díez et al., 2009); as such, it is an important component of any community quality assessment, under the WFD.

FC: Faunal cover. The term FC consists of three components (Pf, Ch and Cs). Pf is the percentage of faunal cover, with respect to the whole benthic community cover (invertebrates plus algae). An increase in invertebrate populations, related to algal stand declines, is a characteristic feature of benthic ecosystems as the organic enrichment derived from domestic sewage implies an additional source of food for fauna (Johnston, 1971; Wilkinson et al., 1987; Kautsky et al., 1992; Roberts et al., 1998; Pagola-Carte and Saiz-Salinas, 2001; Mangialajo et al., 2008). Moreover, the trophic structure of rocky invertebrate assemblages is altered typically in response to changes in environmental conditions (Jones, 1973; Roth and Wilson, 1998; Boaventura et al., 1999; Bonsdorff and Pearson, 1999). Thus, the herbivores cover (Ch) and the suspensivores cover (Cs) were selected also in the development of the metric.

The index ranges between 0 (the worst status) and 1 (the best status). The delimiting boundaries between the five status classes are (see Table 1): Bad: 0–0.2; Poor: >0.2–0.4; Moderate: >0.4–0.6; Good: >0.6–0.8; and High: >0.8–1 (following the same boundaries as in Wells et al. (2007)).

2.7. Index validation using an independent dataset

In order to test the sensitivity of RICQI to detect differences in water quality, we used an independent dataset (calibration dataset, *sensu* Borja and Dauer (2008)) collected at four sites (Muriola, Astondo, Errotatxu and Isla Pobre), located in Plentzia Bay (Fig. 1). Sampling surveys were carried out under three different degrees of anthropogenic pressures, during the gradual implementation of a sewerage scheme: (i) before the construction of the wastewater treatment plant (WWTP) (year 1997); (ii) after the set-up of the primary treatment (2003 and 2005); and (iii) after the implementation of the biological treatment (2007 and 2009). Eighteen quadrats (40 cm × 40 cm) were sampled at the low intertidal zone (0.5–1.3 m), on flat or slightly sloped bedrock platforms. A non-destructive sampling strategy was implemented, which consisted of visually assessed estimates of algal and animal cover (as a percentage) at specific levels, following the scale proposed by Braun-Blanquet (1951). For each site and year, an average inventory was calculated. Similarities between the average inventory of the sites under study and the five reference inventories (corresponding to the five ecological status levels) were measured applying the Bray–Curtis similarity index, to calculate the ESS component of RICQI. The remaining components of RICQI were estimated also. The intertidal assemblages were expected to undergo increases in the abundance of sensitive species, MCA, R as well as decreases in FC and changes in the proportions of different trophic groups, following the pollution removal associated with the sewage treatment. These responses should be reflected by an improvement in the ecological status.

2.8. Comparison with other indices

As the CFR index is being used presently as the official method for assessing the ecological status of macroalgae in the Atlantic coastal waters of Spain (European Commission, 2008), a comparison between RICQI and CFR has been undertaken. The CRF is based upon the assumption that the cover and the number of characteristic algal species decline along a pollution gradient, whereas the

Table 1
The metric scoring system of RICQI. Scores for bioindicator species SpBio (ESS: Ecological Status Similarity; PC: presence/absence of *Cystoseira* genus), morphologically complex algae MCA, species richness R (Ra: algal species; Rf: invertebrates species), and parameters related to faunal cover FC (Pf: ratio faunal cover to whole assemblage cover, Ch: herbivores cover; Cs: suspensivores cover).

	RICQI index score system			
	ESS	Score	PC	Score
SpBio (ESS + PC = max. 0.5)	Bad	0.10	Present	0
	Poor	0.20	Absent	−0.05
	Moderate	0.30		
	Good	0.40		
	High	0.50		

	RICQI index score system	
	MCA	Score
MCA (max. 0.20)	0–15%	0.05
	>15–30%	0.10
	>30–45%	0.15
	>45%	0.20

	RICQI index score system			
	Ra	Score	Rf	Score
R (Ra + Rf = max. 0.15)	0–10	0.02	0–5	0
	>10–20	0.04	>5–10	0.01
	>20–30	0.06	>10–15	0.02
	>30–40	0.08	>15–20	0.03
	>40	0.10	>20–25	0.04
			>25	0.05

	RICQI index score system					
	Pf	Score	Ch	Score	Cs	Score
FC (Pf + Ch + Cs = max. 0.15)	0–5%	0.03	0–5%	0	0–10%	0.05
	>5–10%	0.05	>5%	0.05	>10%	0
	>10–15%	0.04				
	>15–20%	0.02				
	>20–25%	0.01				
	>25%	0				

	RICQI index score system				
	Bad	Poor	Moderate	Good	High
Ecological quality RICQI (SpBio + MCA + R + FC)	0–0.2	>0.2–0.4	>0.4–0.6	>0.6–0.8	>0.8–1.0

fraction of the total algal community (made up by opportunistic algae) increases under anthropogenic pressure. The data collected in the Abra of Bilbao and Plentzia Bay were used to compare both indices. The CFR index was calculated for each site and sampling year, by considering the intertidal macroalgal list and the intertidal scoring criteria (Table 2); there were established for each of the three indicators that make up this index (coverage and richness of characteristic species and opportunist species abundance), according to Juanes et al. (2008) and the European Commission (2008).

3. Results

3.1. Reference conditions

The MDS diagram shows the spatial and temporal relationships between all of the sites studied, between 1996 and 2009 (Fig. 2). The period 1984–1996 was not included in the analysis, because of the lack of quantitative data on invertebrates. The most degraded site (Arrigunaga) lies to the right of the MDS diagram, whereas Azkorri, Meñakoz and Matxilando are located between the most

Table 2
CFR quality thresholds for richness (number) and cover of the characteristic macroalgae population and opportunistic species, in different intertidal types (semiexposed/exposed).

CFR: Quality of rocky bottoms index										
Cover[a]			Populations richness[b]			Opportunistic species[c]		CFR Score	EQR	Status
Score	Semiexp.	Exp.	Score	Semiexp.	Exp.	Score	Exp./Semiexp.			
450	70–100%	50–100%	200	>5	>3	350	<10%	808–1000	0.808–1	High
350	40–69%	30–49%	150	4–5	3	250	10–19%	568–808	0.568–0.808	Good
200	20–39%	10–29%	100	2–3	2	150	20–29%	329–568	0.329–0.568	Moderate
100	10–19%	5–9%	50	1	1	50	30–69%	89–329	0.089–0.329	Poor
0	<10%	<5%	0	0	0	0	70–100%	0–89	0–0.089	Bad

(adapted from Juanes et al. (2008))
[a] % Cover of characteristic macroalgae (CM).
[b] Characteristic macroalgae populations richness.
[c] Relative cover of opportunistic or pollution indicator species respect to the total vegetated surface.

degraded site and the reference conditions (Fig. 2). The displacement of each site, with respect to its initial position, reflects the changes in community composition, over time. A net movement of all of the disturbed sites, towards the reference conditions, can be seen clearly over the time period, reflecting the improvement in water quality. The pollution gradient was divided into four levels of quality, under the WFD: High, Good, Moderate and Poor (Bad is considered as extreme degradation, present over the area prior to 1996). For each quality level the average inventory of flora and fauna, taking into account the samples within each range, was calculated. Among the 237 species recorded, those that exceeded 1% cover (40 taxa) in at least one of the five potential ecological status were selected (Table 3). These 40 taxa were considered to be the indicator species, from bad to high ecological conditions. The Spearman correlation, between the matrix derived from the full species dataset and that obtained from the selected species, is $r = 0.925$, with a significance level of 0.1%, i.e. the spatial-temporal distribution model of communities is very similar. In the case of Bad ecological status, corresponding to situations recorded in the *Abra of Bilbao* bay before 1996, a theoretical inventory was defined, based upon the biological information available prior to 1996.

3.2. Biotic and abiotic relationships

The divisions of the four quality levels, along the pollution gradient of Fig. 2, were based mainly upon expertise knowledge on the ecology of the assemblages. In addition, environmental variables were explored, in order to examine if the suggested divisions were related to the quality levels. Fig. 3 shows the average values of the abiotic variables, for the sites and years within each of the theoretical divisions proposed. A clear increasing trend, from High to Poor status is observed for all of the variables studied. LEC and TUb show significant differences ($p < 0.05$ and $p < 0.01$), between most of the ecological status levels (Table 4). The remaining variables present differences between High and Poor status, with the exception of IMs. On the other hand, the PCO analysis relates the distribution of the assemblages, to the environmental variables (Fig. 4). The first two axes explained 47.8% of the total variation. The two-dimensional plot shows a clear separation of samples, from High to Poor status. In all cases, environmental variables are correlated with PCO 1, whereas no relationships are related to PCO 2. Poor and Moderate ecological status levels are correlated positively with environmental variables, which indi-

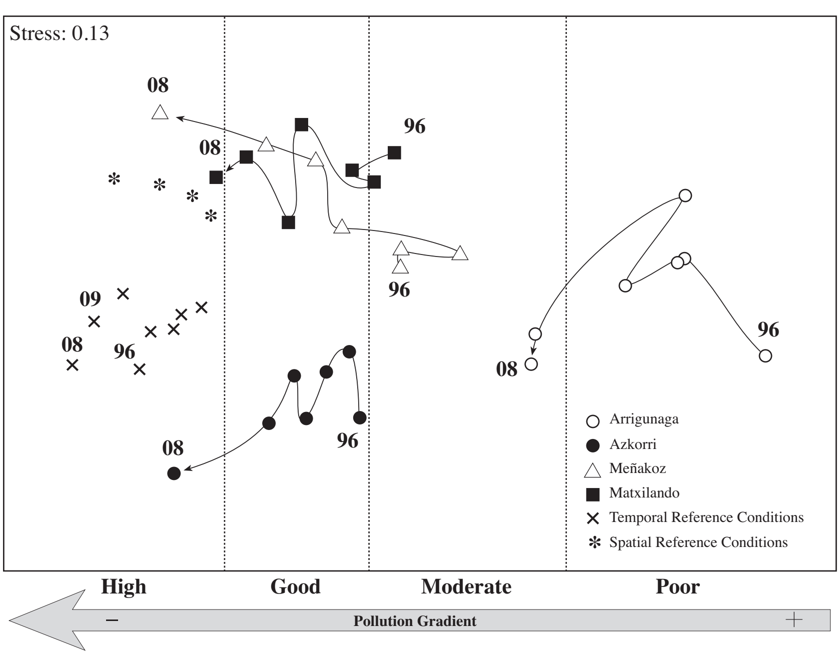

Fig. 2. Non-metric multidimensional scaling ordination plot, based upon species cover, showing separation of assemblages according to sites and time of sampling. The lines reflect the displacement of each site, with respect to its initial position (from 1996 to 2008). Dotted lines separate the pollution gradient into four levels of degradation. Temporal reference conditions (Kobaron). Spatial reference conditions (San Juan de Gaztelugatxe, Berriatua, Itziar and Jaizkibel.

Table 3
Average cover (in %) of indicator species for each of the quality levels (High, Good, Moderate, and Poor) differentiated in the nMDS ordination analysis, on the basis of Bray–Curtis dissimilarity matrix calculated for 4th root-transformed data. A theoretical inventory, based upon data from the area, prior to 1996, represents the bad conditions.

Species list	High	Good	Moderate	Poor	Bad
Bachelotia antillarum	–	–	–	0.6	6.3
Bifurcaria bifurcata	6.2	1.0	–	–	–
Boergeseniella thuyoides	2.4	–	–	–	–
Caulacanthus ustulatus	1.2	4.8	12.2	13.0	1.6
Ceramium botrycarpum	0.5	0.3	3.6	6.4	–
Ceramium ciliatum	0.5	1.6	10.5	10.5	1.3
Ceramium flaccidum	0.3	0.1	6.8	5.4	1.3
Chondracanthus acicularis	3.6	4.0	0.4	–	–
Chondria coerulescens	0.7	5.0	5.6	4.0	–
Chthamalus spp.	1.2	1.3	1.4	–	–
Cladophora lehmanniana	0.8	0.4	2.9	1.5	–
Cladostephus spongiosus	1.4	0.2	0.3	0.6	0.1
Codium decorticatum	0.3	10.7	5.6	0.6	–
Corallina elongata	37.3	51.8	54.5	6.3	–
Cystoseira tamariscifolia	5.8	–	–	–	–
Dictyota dichotoma	0.2	0.1	1.0	1.0	–
Falkenbergia rufolanosa	5.5	2.7	1.2	0.1	–
Gastroclonium reflexum	0.1	0.3	0.3	3.6	–
Gelidium spinosum	1.8	7.2	5.6	–	–
Gelidium pulchellum	0.1	1.9	1.0	1.1	–
Gelidium pusillum	–	0.1	1.6	38.2	48.5
Gymnogongrus griffthsiae	–	–	0.1	0.1	1.1
Hyale spp.	1.0	0.4	0.2	–	–
Hypnea musciformis	1.0	0.4	0.4	–	–
Jania rubens	3.7	0.7	0.1	–	–
Laurencia obtusa	7.9	0.7	–	–	–
Lithophyllum incrustans	10.7	7.9	1.1	–	–
Mesophyllum lichenoides	4.3	10.0	6.8	–	–
Mytilus galloprovincialis	0.5	0.7	2.1	8.6	0.5
Ophidocladus simpliciusculus	1.0	0.1	–	–	–
Paracentrotus lividus	3.5	0.3	0.3	0.1	–
Patella spp.	5.2	2.9	1.0	0.8	–
Plocamium cartilagineum	1.1	0.8	–	–	–
Polydora spp.	–	–	1.0	27.0	37.5
Polysiphonia atlantica	–	–	0.0	0.6	1.1
Pterosiphonia complanata	1.7	6.1	1.9	–	–
Pterosiphonia pennata	0.7	1.0	0.5	0.5	–
Ralfsia verrucosa	1.4	1.4	2.2	0.2	–
Stypocaulon scoparium	32.2	11.0	3.0	–	–
Ulva rigida	0.7	4.6	12.5	3.4	2.0

cate low water quality. The abiotic variables with a greater vector length are: light extinction coefficient (LEC = 0.8); turbidity at the surface (TUs = 0.7); turbidity at the bottom (TUb = 0.8); and inorganic matter at the bottom (IMb = 0.6). These variables highlight the relationships of the proposed divisions of assemblages, with the environmental water condition.

3.3. Index functioning

From the different metrics used in this index, the SpBio consists of two components (ESS and PC) and is developed from the abovementioned reference conditions (Table 1). If the highest similarity corresponds to the Bad status inventory the ESS component is 0.1; 0.2 for Poor; 0.3 for Moderate; 0.4 for Good; and 0.5 for High. Besides, the PC component acts as a correction factor and, if the genus *Cystoseira* is absent in the area under assessment (not necessarily in the quadrats sampled, but considering the whole area), 0.05 is subtracted from the SpBio term.

Fig. 5 shows the variation in the abundance of calcareous (articulated plus crustose species), morphologically simple forms (uniseriate, polysiphonous, foliose non-corticated and slightly corticated: cortex with one-two layers) and complex algae (corticated algae: cortex with more than two layers, plus leathery macrophytes), during the recovery process and used as reference information to develop the RICQI. Under severely altered conditions (e.g. Arrigunaga, from 1984 to 2004), morphologically simple forms are the main components of the vegetation; as pollution decreases, these are replaced by calcareous algae and, finally, coarse and leathery species enter into the communities. Although morphologically simple forms are more abundant at the disturbed sites, than at the reference stations (Fig. 5A), they are not relevant in distinguishing between moderately degraded and unaltered vegetation. Calcareous algae, for example, are nearly absent when the environmental conditions are altered strongly but reach their highest covers under moderate levels of pollution (Fig. 5B). MCA (Fig. 5C) show the best response to changes in water quality levels, since they increase progressively as pollution decreases. Therefore, only the MCA data were used to develop the RICQI, since they have the best bioindicator attributes. Quality thresholds for this component are shown in Fig. 5C and listed in Table 1.

The quality thresholds for algal and invertebrate species richness, as used in the RICQI, are shown in Fig. 6A and B and Table 1. The Ra shows a clear increase from 1984 onwards, at all of the disturbed sites: Arrigunaga (8 in 1984 *vs.* 30 in 2008); Azkorri (20 *vs.* 44); Meñakoz (25 *vs.* 39); and Matxilando (29 *vs.* 57). Generally, it shows values of more than 40 species under the reference conditions.

Low values of Rf (Fig. 6B) are recorded under severe pollution levels, where the degraded sites show an increase over the period of the study: Arrigunaga (6 in 1992 *vs.* 19 in 2008); and Azkorri (20 *vs.* 28). The two remaining sites under the recovery process,

Table 4
One-way PERMANOVA results for the main test (*F*) and pair-wise comparisons (*t*) between ecological status (H: High; G: Good; M: Moderate; P: Poor) for the environmental variables analysed: light extinction coefficient (LEC), turbidity at the surface (TUs) and bottom (TUb) layers, total suspended solids at the surface (TSSs) and bottom (TSSb) layers, organic matter at the surface (OMs) and bottom (OMb) layers, inorganic matter at the surface (IMs) and bottom (IMb) layers.

Variable			H *vs.* G		G *vs.* M		M *vs.* P		H *vs.* M		H *vs.* P		G *vs.* P	
	F	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
LEC	31.230	**	2.6091	*	2.6603	*	3.6387	*	4.4994	**	8.2069	**	7.3264	**
TUb	13.923	**	1.1529	n.s.	3.5554	*	2.1860	*	3.8334	**	4.0785	**	4.4700	**
TUs	23.449	**	3.4754	**	0.9110	n.s.	2.8950	n.s.	1.9946	*	18.060	*	10.7270	**
TSSb	9.1592	**	1.5607	n.s.	0.4879	n.s.	2.7005	n.s.	2.1650	*	5.4410	**	3.6237	**
TSSs	2.9710	n.s.	0.5260	n.s.	1.0051	n.s.	0.9776	n.s.	1.6047	n.s.	2.2201	*	2.1287	n.s.
IMb	14.010	**	1.0959	n.s.	1.7579	n.s.	2.4885	n.s.	2.9644	*	5.5678	**	4.8398	*
IMs	3.0101	n.s.	0.1150	n.s.	1.3926	n.s.	0.9325	n.s.	1.5017	n.s.	2.0644	n.s.	2.2288	n.s.
OMb	4.5659	*	0.3495	n.s.	1.3805	n.s.	1.5711	n.s.	1.9424	n.s.	4.2041	**	2.9599	*
OMs	4.2452	*	1.4617	n.s.	0.5563	n.s.	1.2977	n.s.	1.7589	n.s.	2.9717	*	2.4016	*

n.s. – no significant.
* $p < 0.05$.
** $p < 0.01$.

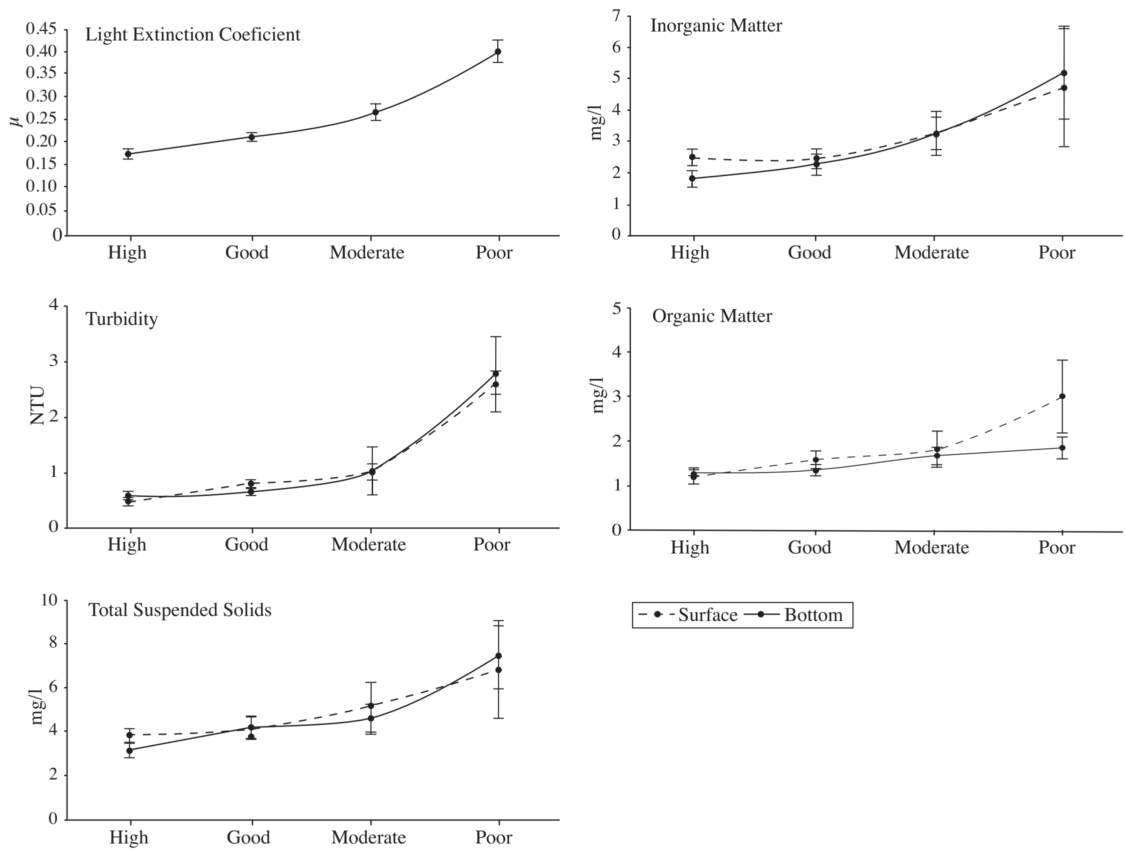

Fig. 3. Variation in environmental variables (mean and standard error), according to the four quality levels distinguished from the assemblage MDS ordination (see Fig. 2). Key: LEC = light extinction coefficient; TU = turbidity; TSS = total suspended solids; OM = organic matter; IM = inorganic matter; at surface and bottom of the water column.

Meñakoz and Matxilando, do not follow a clear pattern during the pollution removal process. In general, the reference condition sites show higher values than the other sites. Quality thresholds for this metric are shown in Table 1.

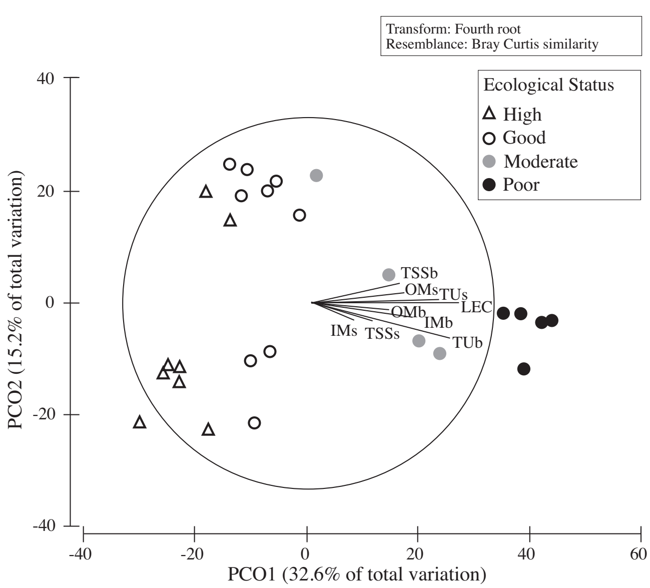

Fig. 4. Principal coordinates analysis, including both biological and environmental variables. Key: LEC = light extinction coefficient; TU = turbidity; TSS = total suspended solids; OM = organic matter; IM = inorganic matter; at surface (s) and bottom (b) of the water column.

Fig. 7 shows the variation in the relative abundance of fauna with respect to the benthic community (flora plus fauna) and the variation in the abundance of suspensivores and herbivores, during the recovery process in the Abra of Bilbao. The quality thresholds are listed in Table 1. The Pf was highest at the degraded sites, with values decreasing over the period of the study: Arrigunaga (39% in 1992 *vs.* 6% in 2008); Azkorri (19% in 1996 *vs.* 5% in 2008); Meñakoz (13% *vs.* 3%); and Matxilando (7% *vs.* 2%) (Fig. 7A). In general, extremely low percentages of fauna with respect to the benthic community are recorded at sites under low levels of pollution (Pf: 0–5%); intermediate values (Pf: 5–15%) appear at the reference sites. With respect to trophic guilds, the highest Ch values are obtained at the reference sites (Fig. 7B), whereas sites under the recovery process show lower values (excluding Azkorri). Conversely, Cs maintains low values at the reference sites and at intermediate pollution levels (Fig. 7C). At the most stressed sites high abundance of suspensivores is found, with all of the sites declining over time: Arrigunaga (38% in 1992 *vs.* 7% in 2008); Azkorri (19% in 1996 *vs.* 4% in 2008); Meñakoz (18% *vs.* 1%); and Matxilando (2% *vs.* 1%).

3.4. Metrics integration

The scores obtained from each of the metrics are added together, to produce the final quality status. It should be noted that a distinct specific weight is given to each of the RICQI metrics (SpBio contributes up to 50%, MCA up to 20%, R 15% and FC 15%) because the sensitivity of each metric varies, reflecting changes in environmental quality. Therefore, the SpBio shows the clearest response to disturbance, this is followed by the abundance of MCA, and then species richness and fauna cover. Unfortunately, there is only lim-

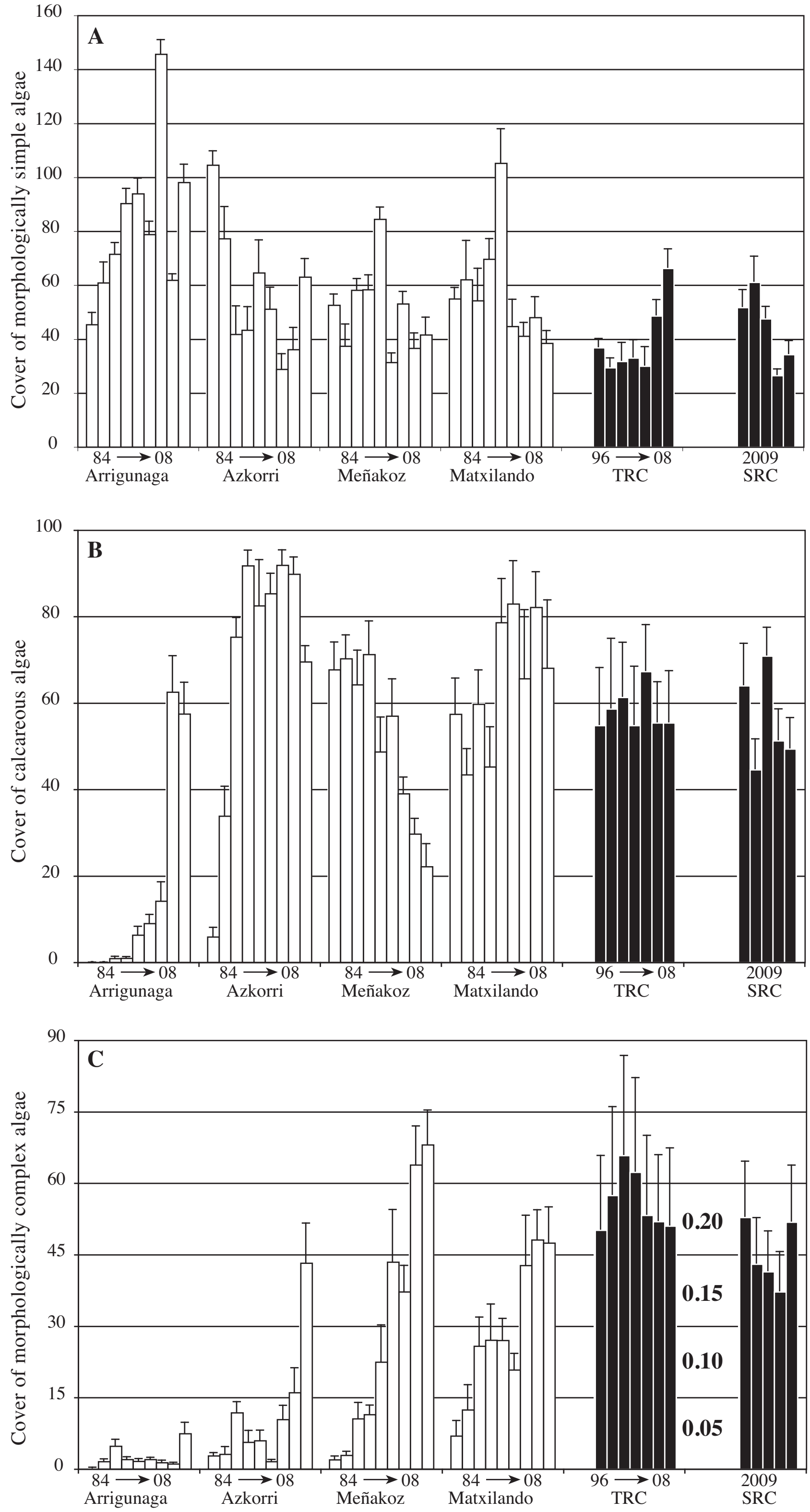

Fig. 5. Cover (in %) and standard error (SE) of morphologically simple algae (A), Calcareous algae (B), and morphologically complex algae (C), through time at degraded conditions and at reference conditions. Key: TRC: temporal reference condition; and SRC: spatial reference condition.

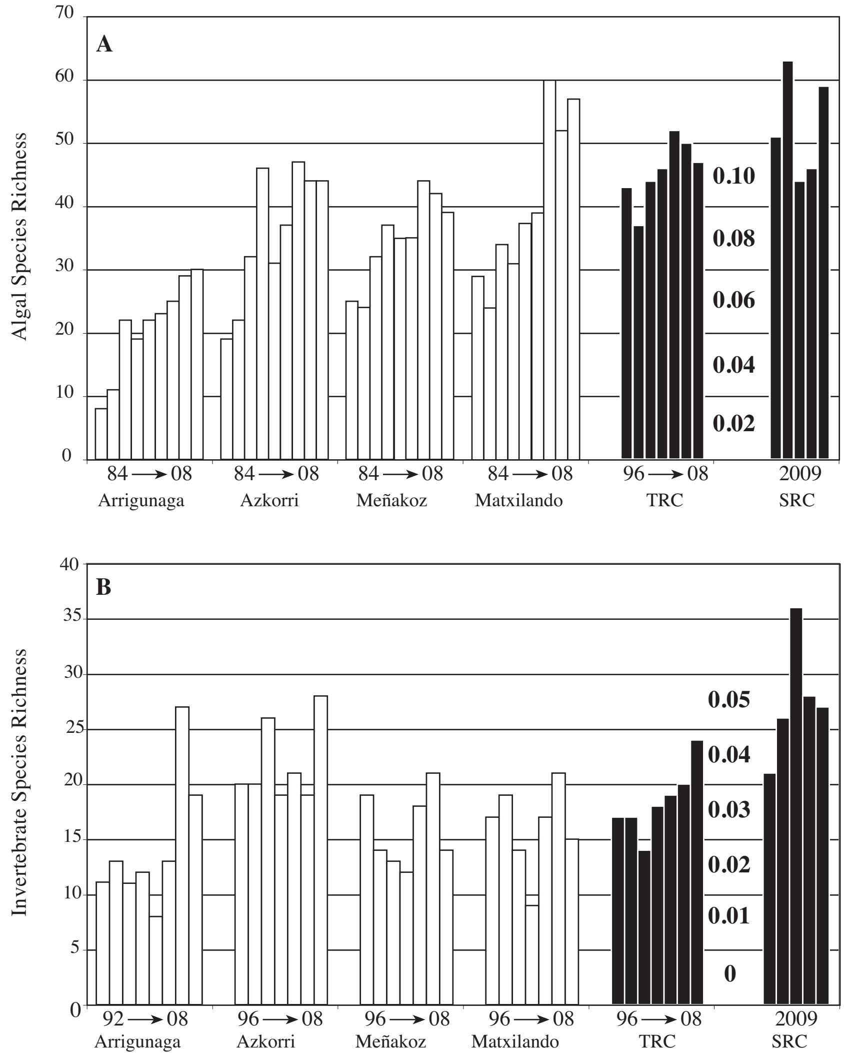

Fig. 6. Algal species richness (A) and invertebrate species richness (B), over time, at degraded conditions and at reference conditions. Key: TRC: temporal reference condition; SRC: spatial reference condition.

ited physicochemical data to supplement the criterion used, which is based largely on expert knowledge.

3.5. Index validation using an independent dataset

The results obtained suggest that RICQI responds clearly to changes in water quality in Plentzia Bay (Table 5). Hence, prior to the set-up of the WWTP, the best ecological conditions in the bay were found at Muriola, the site farthest from the influence of the outfall and the mouth of the river Butroi (Fig. 1). Assemblages at this site recorded Good ecological status throughout the study (Table 5).

Table 5
Quality values obtained by applying RICQI in the assessment of ecological status (H: High; G: Good; M: Moderate; P: Poor) of rocky intertidal communities at four stations located in Plentzia Bay prior to the set-up of a Waste Water Treatment Plant (1997), during the application of a primary treatment (years 2003 and 2005), and after the addition of the biological treatment (years 2007 and 2009).

	Pre-operational	Primary treatment		Biological treatment	
	1997	2003	2005	2007	2009
Muriola	G (0.73)	G (0.71)	G (0.77)	G (0.78)	G (0.74)
Astondo	M (0.47)	M (0.44)	M (0.58)	G (0.70)	G (0.60)
Isla Pobre	P (0.37)	M (0.49)	M (0.59)	G (0.70)	G (0.62)
Errotatxu	M (0.53)	M (0.59)	G (0.72)	G (0.75)	G (0.63)

Benthic communities at Astondo were classified as Moderate ecological status, from 1997 to 2005; they were assessed as Good status after the improvement of the WWTP, through the introduction of biological treatment. In the pre-operational stage, communities at Isla Pobre, the station that receives directly the effluent, were assessed as Poor ecological status (Table 5). The assessment moved to Moderate status with the introduction of primary treatment (2003) and, finally, to Good status with the application of biological treatment (2005). The Errotatxu communities moved from Moderate to Good ecological status (2005).

3.6. Comparison with other indices

The quality values obtained by applying the CFR index, to the same rocky intertidal communities used to develop the RICQI, are listed in Table 6. Similar to RICQI, the CFR index showed High ecological status for the reference site, in the absence (or limited presence) of human pressure. However, CFR gave high scores at assemblages under the recovery processes, even at early stages of recovery. In 1996, the CFR index shows High ecological status in Azkorri, Meñakoz and Matxilando, with Good ecological status in Arrigunaga.

In the period before the WWTP came into operation in Plentzia Bay, the CFR index identified the lowest ecological conditions at

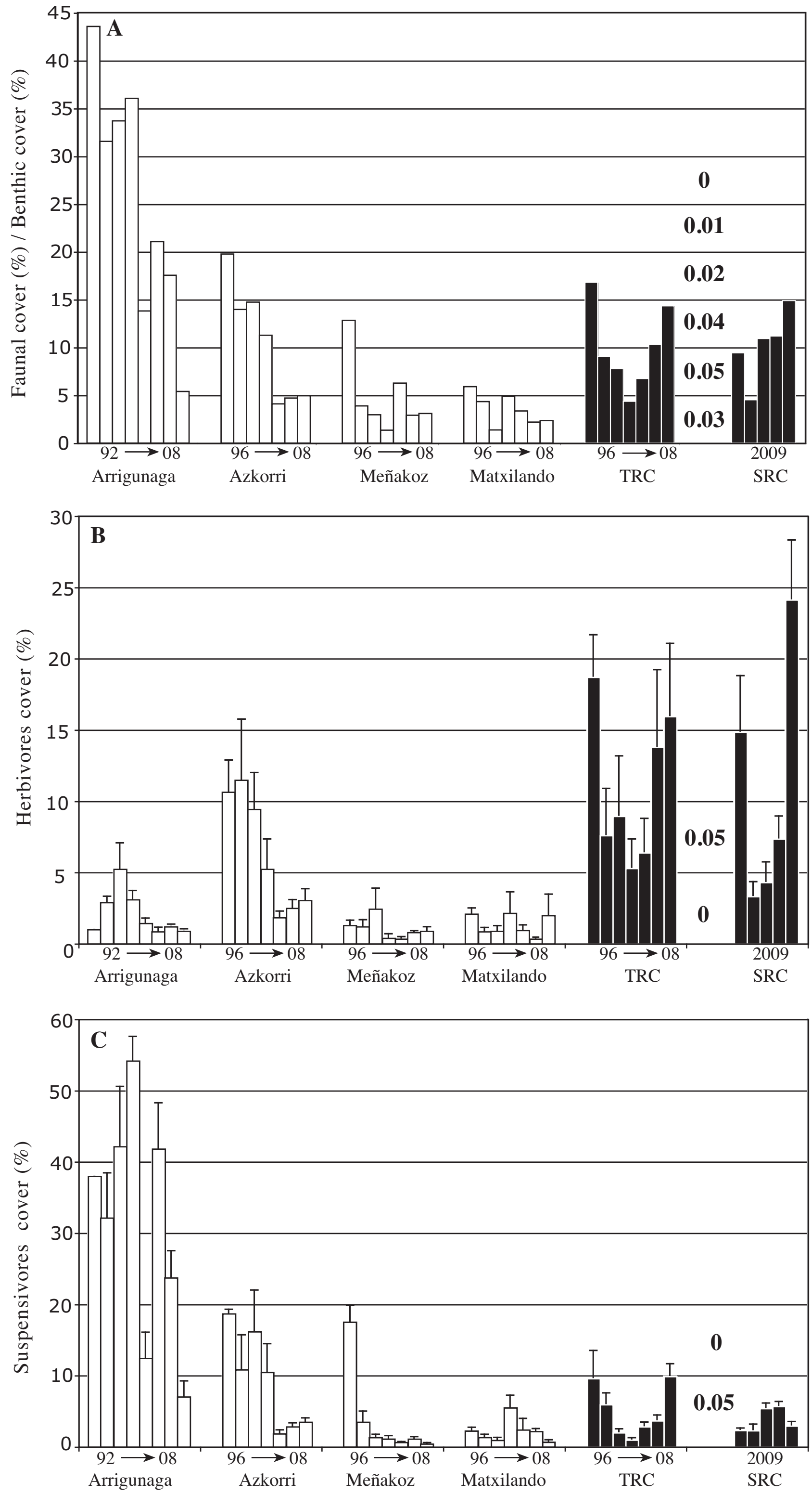

Fig. 7. (A) Percentage of faunal cover with respect to benthic community, together with cover (in %) and standard error (SE) of herbivores (B) and suspensivores (C), over time, at degraded conditions and at reference conditions. Key: TRC: temporal reference condition; SRC: spatial reference condition.

Isla Pobre (the station that receives directly the effluent) (Table 6). However, some inaccurate results are detected when the CFR is used. On the one hand, communities at Isla Pobre show High ecological status, even before biological treatment comes on line. The same result is obtained for Errotatxu. On the other hand, in the pre-operational period, the communities at Astondo show High ecological status. This site was affected less by the outfall than Isla Pobre, but it received directly water from the river Butroi, carrying pollutants from various anthropogenic activities upstream. In addition, quality scores were lower during the primary treatment stage than in the pre-operational state at Astondo. With the exception of Muriola in 2005, the CFR index (Table 6) gives higher scores in

Table 6
Quality values obtained by applying the CFR index to the assessment of ecological status (H: High; G: Good; M: Moderate; P: Poor) of rocky intertidal communities at the sampling sites adjacent to *Abra of Bilbao* (used to develop the RICQI), and in Plentzia Bay (independent dataset used for index validation). Key: DS: Degraded Sites; TRC: temporal reference conditions.

Adjacent coast to Abra of Bilbao	Recovery process						
	1996	1998	2000	2002	2004	2006	2008
DS							
Arrigunaga	G (0.80)	M (0.40)	P (0.25)	G (0.65)	M (0.40)	G (0.70)	G (0.70)
Azkorri	H (0.85)	H (0.85)	H (0.85)	H (0.85)	H (0.95)	H (0.90)	H (0.90)
Meñakoz	H (0.85)	G (0.75)	G (0.75)	H (1.00)	H (0.90)	H (1.00)	H (0.90)
Matxilando	H (0.95)	H (0.85)	G (0.75)	G (0.80)	H (0.95)	H (0.85)	H (0.95)
TRC							
Kobaron	H (1.00)	H (1.00)	H (1.00)	H (1.00)	H (1.00)	H (1.00)	H (1.00)

Plentzia bay	Pre-operational	Primary treatment		Biological treatment	
	1997	2003	2005	2007	2009
Muriola	G (0.80)	H (0.85)	G (0.75)	H (0.95)	H (0.85)
Astondo	H (0.95)	H (0.85)	G (0.70)	H (0.90)	H (0.90)
Isla Pobre	G (0.65)	G (0.80)	H (0.95)	H (0.90)	H (0.90)
Errotatxu	G (0.80)	G (0.85)	H (0.95)	H (0.95)	H (0.85)

the ecological assessment of intertidal communities, than the RICQI (Table 5). In summary, CFR is a less sensitive tool, than RICQI, for assessing the ecological quality of intertidal assemblages.

4. Discussion

The assessment of ecological status plays an important role in the management of coastal zones; however, only a small number of ecological indices are applicable to rocky bottoms (Mangialajo et al., 2007; Borja et al., 2012). In this study, the ecological quality status obtained using the RICQI shows that the method responds to the human pressure produced by a sewerage scheme. Assemblages that received directly wastewaters from the effluent, moved from Poor ecological status to Moderate status upon the introduction of primary treatment; they moved further to Good status, with the implementation of the biological treatment.

The sensitivity of the RICQI is based upon the establishment of a classification system using the composition and structural characteristics of rocky intertidal communities. A conceptual model of the community successional stages along a gradient of increasing disturbance is proposed (Fig. 8). From High to Bad ecological status, species composition, species richness, cover of MCA and fauna cover change as summarised here, according to the normative definitions within the WFD: (i) High – The large perennial macrophytes *Cystoseira tamariscifolia, Bifurcaria bifurcata, Stypocaulon scoparium,* and *Gelidium spinosum* occupy the lowest intertidal level. Landwards, *Corallina elongata* shares substratum with caespitose forms such as *Laurencia obtusa* and *Chondracanthus acicularis* and crustose calcareous algae. The invertebrates *Patella* spp. and *Paracentrotus lividus* are abundant. Species richness, MCA and herbivorous cover show the highest levels. (ii) Good – *C. tamariscifolia* is absent. Cover of *B. bifurcata, L. obtusa* and *C. acicularis* decreases significantly. The calcareous *C. elongata* becomes dominant. *S. scoparium* and *G. spinosum* remain abundant. The urchin *P. lividus* decreases significantly, whereas *Patella* spp. remains abundant. MCA and herbivorous cover decrease, whilst species richness may be altered. (iii) Moderate – *C. elongata* remains dominant, showing its highest cover. The turfing algae *Caulacanthus ustulatus* and *Ceramium* spp. become abundant, whilst *L. obtusa* and *C. acicularis* disappear. Crustose calcareous algae show low covers. Species richness and MCA decrease, whereas invertebrates, essentially suspensivores such as *Mytilus galloprovincialis*, increase. (iv) Poor – *Gelidium pusillum* and *Polydora* spp. become dominant, whilst *C. ustulatus*, *Ceramium* spp. and *M. galloprovincialis* are abundant. Species richness decreases, the percentage of substratum occupied by fauna increases significantly, essentially suspensivores. (v) Bad – dominated by *G. pusillum*, *Bachelotia antillarum* and *Polydora* spp. Species richness and MCA reach minimum values, whilst invertebrates, essentially suspensivores, are abundant.

The underlying structure of the benthic community succession proposed in this conceptual model is consistent broadly with the alterations reported for warm-temperate assemblages, under human pressures. Therefore, there is ample evidence that coralline algae tend to become dominant when *Cystoseira* species are lost, as a consequence of anthropogenic disturbances (Benedetti-Cecchi et al., 2001; Arévalo et al., 2007; Mangialajo et al., 2008; Bertocci et al., 2010). In general, the loss of perennial canopy-forming algae is considered to be the first signal of vegetation degradation (Brown et al., 1990; Eriksson et al., 2002; Connell et al., 2008). It has been documented also that, as disturbance increases, species of *Corallina* are replaced progressively by small fast-growing species more tolerant to pollution, such as *G. pusillum* (May, 1985; Brown et al., 1990) and other simple forms (Pinedo et al., 2007). Likewise, chronic domestic pollution encourages the development of filter-feeding animals, particularly mussels and barnacles; these take advantage of the organic matter enrichment (Bellan and Bellan-Santini, 1972; Kautsky et al., 1992; Pinedo et al., 2007). In agreement with the results obtained here, the replacement of phytobenthic assemblages, by extensive cover of polychaetes in response to severe pollution, has been reported also in other European areas (Wilkinson et al., 1987).

The use of functional-form group models, as a tool to predict changes in algal community structure resulting from disturbance has been proposed by several authors (Littler and Littler, 1980; Steneck and Dethier, 1994). Therefore, other multimetric quality indices, such as the EEI (Orfanidis et al., 2001) and the RSL index (Wells et al., 2007), include functional-form groups as indicators of environmental health. Both these methods classify species into two ecological state groups (ESG): ESG 1, which includes calcareous, highly corticated and leathery forms (late successionals and perennials); and ESG 2, which includes unicellular, filamentous, sheet-like and slightly corticated forms (opportunists and annuals). According to our results, although severely disturbed and pristine communities clearly show differences in the abundance of ESG 2, this information appears to be less discriminating in differentiating between moderate and slightly disturbed assemblages. Similarly, calcareous algae, that show the highest covers under moderate levels of pollution, are of little use in differentiating between moderate and slightly disturbed environments. In contrast, the abundance of MCA seems to provide excellent information, because these

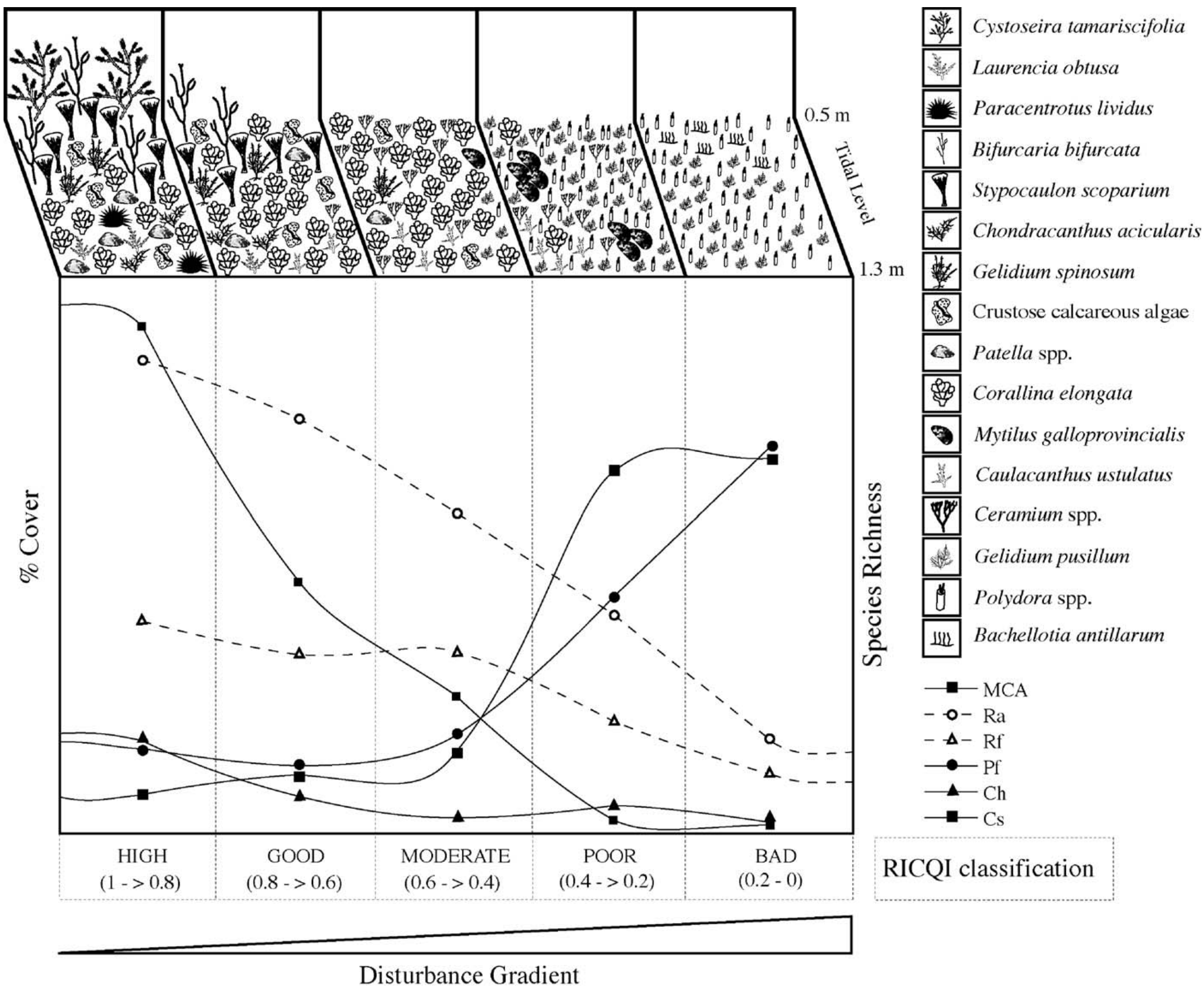

Fig. 8. Conceptual model proposed for successional stages along a gradient of increasing environmental disturbance and associated values of metrics included in the index. Key: MCA: Morphologically complex algae; Ra: algal species richness; Rf: invertebrate species richness; PF: faunal percentage with respect to benthic community; Ch: herbivores cover; and Cs: suspensivores cover.

macrophytes increase progressively from severely disturbed to pristine conditions.

The shift in the community composition, from specialised to opportunist species along the disturbance gradient, is accompanied by a decrease in species richness. Loss of species richness is a common factor in the measurement of anthropogenic disturbance (Littler and Murray, 1975; May, 1985; Tewari and Joshi, 1988; Munda, 1993; Roberts, 1996; Underwood and Chapman, 1996; Arévalo et al., 2007; Wear and Tanner, 2007). Similarly, increases in the number of taxa following water quality improvements have also been reported (Bonk et al., 1996; Gorostiaga and Díez, 1996; Archambault et al., 2001; Soltan et al., 2001). Nevertheless, other studies do not find a significant effect of disturbance on the total number of species (Terlizzi et al., 2002, 2005). These discrepancies may be related to the frequency and intensity of disturbance, as well as in the disparity of the physical environmental conditions in which each study is undertaken. Our results reveal that species richness decreases significantly under heavily altered conditions, particularly the number of algal species. It drops also in moderately degraded communities, although it is not a discriminating measure between slightly degraded assemblages and unaltered ones. It could be inferred from some cases of the present study, that low levels of disturbance could promote the introduction of new species, by preventing the competitive equilibrium of mature communities. As result of this, species richness has been used as a metric in the development of RICQI but with a lower specific weight than the metrics SpBio and MCA.

None of the available ecological indices for rocky substrata incorporate fauna. In this sense, RICQI is not fully compliant with WFD requirements, since macroalgae and invertebrates are not treated as different BQEs. However, other authors have recommended also considering the simultaneous use of both fauna and flora for the determination of the ecological status of hard substrata (Hiscock et al., 2005). The results presented here show that invertebrates are implicitly intricate in the degrees of alteration of macroalgae, since a progressive loss of algae is associated with the proliferation of fauna, as a consequence of disturbance. Whilst the assessment procedures are still under development, emphasis needs to be placed on comprehending the complexities of coastal systems, instead of simplifying them into smaller components

(Diaz et al., 2004). Seaweeds represent an important biological resource for animals that make use of the algae for food, shelter and support (Hayward, 1988). Over the mid-intertidal, algal turfs allow the presence of many invertebrate species, that otherwise would be absent at the same tidal level (Bejín et al., 2004; Bertness et al., 2006) and influence the ecological patterns of associated animals (Hauser et al., 2008). At the same time, the patchiness that characterises the rocky benthos worldwide is generated mainly by invertebrates (Sousa, 1984; Branch, 1985). The classification of ecological status of rocky substrata is challenging, since algae and invertebrates covary, both naturally and with the alteration of the environmental conditions. Given the close link between flora and fauna the WFD policies might consider extending their definitions regarding BQEs. Otherwise, essential information will be lost in our interpretation of changes, especially in the case of humanly altered ecosystems, as it has been demonstrated in this study.

Two boundaries between the five ecological status levels are especially relevant: (i) between Good and Moderate, as it distinguishes between acceptable and unacceptable levels of quality (according to the WFD); and (ii) between Good and High status. If slightly disturbed assemblages are mistakenly considered to show pristine conditions, environmental management decisions could lead to a progressive loss of biodiversity. Therefore, the main challenge when developing a biotic quality index is to draw up a sensitive tool for detecting differences between moderately, slightly altered and undisturbed communities. In this regard, our results suggest that the RICQI is a more sensitive method than the CFR, for assessing the ecological status of the rocky intertidal communities of the Iberian coast. Discrepancies between the two indices may be due largely to the way in which species sensitivity to pollution is classified. CFR is based mainly upon two species lists: a "characteristic" species list, which includes the most sensitive macrophytes; and an "opportunistic" species list, composed of species tolerant to anthropogenic disturbances. In contrast, RICQI uses five theoretical inventories, with the relative abundance of species varying from the bad status inventory to the high status inventory. As a result, under CFR, *Corallina* spp., *S. scoparium* and *Cystoseira baccata* are considered to be equally tolerant to pollution; under RICQI, they show different degrees of tolerance.

The RICQI has been developed for the Basque Country and, therefore, reference conditions as well as the ecological class boundaries are relevant to this coastal area and may not transpose directly into other regions. Thus, all metrics will have to be intercalibrated subsequently in order to guarantee that metrics assess the same water bodies in the same status.

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Sources of uncertainty in estimation of eelgrass depth limits

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ABSTRACT

In coastal areas seagrasses have considerable ecological importance and they respond to eutrophication pressures. Seagrasses have become an important parameter for assessing ecological status of marine water bodies. In this study we analyzed the sources of uncertainty associated with the monitoring of the max depth limit in eelgrass (Zoestra marina). Based on a long term marine monitoring of eelgrass max depth limit in Danish coastal waters we estimated the uncertainty contribution of year, diver, transect and replicates for each water body. For all variables the uncertainty increased with the maximum depth limit, which suggested that eel grass depth limits were more difficult to determine or less well defined at large depths. We used either a Spheric or a Gaussian function to describe the relation between uncertainty and the max depth limit for each variable. This parameterization of the depth specific uncertainty allowed estimation of the total variance, which can be used to evaluate survey designs. The total variance was compared with the time budget for a survey in a water body. If a maximum time limit was allocated to survey a water body, the surveys that resulted in the lowest variance of the maximum depth limit used 2 divers if 100 h were available and 3 divers if 200h were available, 2 or 3 years of survey and 4 to 8 transects.

INTRODUCTION

Eelgrass and other seagrasses are widely distributed in temperate and tropical coastal waters (Short et al. 2011). The meadows provide habitat for a wealth of organisms, some of which complete their lifecycle there, while others use them as hatching and nursery areas. Seagrass meadows also play important roles in the sequestration of carbon and recycling of nutrients, stabilize the seabottom, and protect coastlines against erosion. The many important ecological functions make seagrasses key components of healthy coastal ecosystems (Hemminga & Duarte 2000).

Seagrasses grow in relatively shallow coastal waters with their maximum depth limits extending as deep as light levels allow their growth to balance losses (Dennison 1987). Seagrass depth limits are, therefore, mainly determined by water clarity (Duarte et al. 2007, Ralph et al. 2007, Krause-Jensen et al. 2011b), which is affected by eutrophication (e.g. Cloern 2001). High nutrient loads stimulate growth rates and hence concentrations of phytoplankton, which reduce water clarity (Nielsen et al. 2002). On a longer term, nutrient pressures may also lead to increased concentrations of suspended particles of mineral and organic origin, which further reduce water clarity (Olesen 1996). Increased sedimentation of organic-rich material may impair sediment quality (Koch 2001, Krause-Jensen et al. 2011a) and increase the risk of water column anoxia and emission of hydrogen sulfide, which hamper seagrasses (Holmer & Bondgaard 2001, Pulido & Borum 2010). Several additional factors unrelated to eutrophication also affect eelgrass distribution in space and over time. Examples are physical disturbance due to natural factors such as wind and wave exposure or human causes such as dredging or anchoring activities (Short & Burdick 1996) as well as variations in e.g. sediment structure (Koch 2001) or temperature (Stæhr & Borum 2011).

The sensitivity of seagrasses to human pressures in combination with their key ecosystem functions make them useful indicators of ecological status and a wide range of seagrass indicators are used in Europe e.g. for assessing ecological status as required by the Water Framework Directive (WFD) (Marbá et al. submitted). The need for assessing the status of seagrasses is further accentuated by the global threats and marked declines of these ecosystems (Waycott et al. 2009). The three most common seagrass indicators are shoot density, cover and depth limit (Marbá et al. submitted). Contrary to measurements of Secchi depth, which provide snapshots of a highly dynamical variable, the depth limit s of perennial seagrasses constitute an integral indicator of light conditions over a much longer time span, and, in addition, describes the distribution boundary of the seagrass habitat.

To estimate the confidence in WFD classification of ecological status and design an optimal monitoring program, we need information on the different sources of variation associated with the assessment. Eelgrass depth limits may, as argued above, vary substantially within and across water bodies, and in order to be able to detect a trend caused by e.g. reduced eutrophication pressure, it is important that the monitoring program is designed to minimize the influence of random variation in space and time as well as the variation due to methodology. Random variation in estimates of depth limits of a given water body can be categorized as year-to-year variation, methodological variation between divers, large-scale spatial variation between individual transects/areas of the water body and smaller scale spatial variation between replicate observations. Quantification of these important sources of variation can devise means to reduce uncertainties related to the monitoring methods and program, and thus improve the precision of the indicator and its power to detect changes.

The depth limit of eelgrass is the main indicator of ecological status of Danish coastal waters where a large historical dataset provides information on the distribution of eelgrass during the last century and serves as a reference on distribution patterns under relatively undisturbed environmental conditions (Krause-Jensen & Rasmussen 2010). A large-scale and long-term monitoring data set on the maximum depth limit of eelgrass in Danish coastal waters has been conducted since 1989. The resulting data set, encompassing two decades, shows no trend in depth limits despite marked reductions in nutrient load, most likely because water clarity has not improved markedly and prevents a return from the current plankton-dominated regime to an eelgrass dominated regime characteristic of the oligotrophic situation in the past (Krause-Jensen et al. 2011b; Hansen & Pedersen 2010; Carstensen et al. this volume). However, the large data set also contains unique information for estimating how much each of the sources of variation associated with the monitoring scheme, i.e. variation between year, diver, transects and replicate observations contribute to the uncertainty in the estimation of the maximum depth limit of eelgrass. Quantification of these sources of variation provide a basis to suggest an optimal design for a program to monitor seagrass depth limits.

This paper has three goals: 1) To quantify the sources of random variation that affect the estimates of eelgrass depth limits. 2) To identify how the uncertainty varies across the range of depth limits. 3) To develop general parametric relationships to describe the magnitude of uncertainty components in a given water body. 4) On the basis of the findings we will illustrate how the identified sources of uncertainty and their magnitudes can be applied to design a cost-efficient monitoring program.

METHODS

Study areas and sampling

The data set on eelgrass maximum depth limit analyzed in the present study has been collected as part of the Danish national environmental monitoring program between 1993 and 2009. The local environmental Authorities conducted the monitoring according to a common set of guidelines. Monitoring was conducted by 29 different divers at a total of 352 transects, distributed over 65 areas from 51 water bodies in Danish coastal waters in the Kattegat-Belt area of the Baltic Sea (Figure 1). Some water bodies consisted of more than one area, e.g. an inner and an outer part of a water body (Table 1). Typically, inner and outer estuaries differed substantially in terms of salinity, eutrophication pressure and hence the maximum depth limit also differed. Therefore we analysed data on basis of area rather than water body.

The monitoring took place between April and September with the majority (78%) conducted in June-August. At each transect a diver estimated the maximum depth limit of eelgrass defined as the deepest occurring shoot. The diver swam from the coast to deeper depths and when reaching the maximum depth limit defined as the deepest shoot, would swim 30 m orthogonal to each side of the transect crossing the depth limit multiple times and recording seven replicates of the maximum depth limit (Figure 1). For each recording, the diver placed the depth sensor on the bottom to read the maximum depth limit. In the following, the maximum depth limit is referred to as depth limit. Usually the same transects were monitored for multiple years, although this was not consistent throughout the entire data set.

With most transects surveyed each year with replicate observations, the data can be considered somewhat hierarchically organized and we treat transect, year, and replicate as random factors. Diver was also included as a random variable although all divers were not used in all years and a specific diver would only survey a subset of the transects within a limited geographical region.

Statistical analysis

We used general linear mixed models (GLMM) to estimate the variance contribution of the different sources of uncertainty on eelgrass depth limits. First, the overall variance contributions of 1) interannual variation, 2) different divers, 3) spatial variation among transects within a subarea, and 4) spatial variation of replicates within a transect in a given year and for a given diver were estimated with a model that included a mean parameter specific to water body and area within that water body ($\mu_{water\_body,area}$):

$$Y_{ijkl} = \mu_{water\_body,area} + A_i + B_j + C_k + e_{ijkl} \qquad \text{(Eq. 1)}$$

where Y_{ijkl} is the eelgrass max depth limit, A_i is the random variation across years with a variance of σ_A^2, Bj is the random variation among divers with a variance of σ_B^2, C_k is the random variation among transects with a variance of σ_C^2 , and e_{ijkl} is the random variation among replicates within transects with a variance of σ^2 . The generality of the four variance components was examined by estimating the same model specifically for each water body and area within water body, thus producing 65 sets of variance estimates. The model did not include interactions between the main factors (A_i, B_j and C_k), since the sparseness of combinations of all three factors did not allow for their estimation. This implies that the random variation among divers was assumed to be the same for all transects and years, and similarly for the interannual variation and the spatial variation among transects. The mean depth limit was a fixed factor, and it did not influence the estimates of the random effect components.

For some areas within water bodies the estimation of the mixed model above (Eq. 1) did not converge (resulting in a non-positive Hessian matrix) due to lack of combinations of the random factors or alternatively, one of the random factors had one level only and therefore its variance could not be estimated. Hence, we removed the constraining factor for those areas within water bodies, where convergence problems were experienced, and re-estimated the model. Estimates for all four variance components were obtained from 58 areas, whereas A_i was removed from 5 areas, B_j was removed from 21 areas, and C_k was removed from 6 areas (cf. Table 1).

Estimates of the variance components were related to the mean depth limit to investigate if the uncertainty was constant or proportional across the expected mean levels, suggesting the data to be normal or lognormal distributed. We also analysed if the nature of the relationship between the standard errors of the random factors (square root of the variances) and the expected max depth limit deviated from a constant or proportional relationship, suggesting a more complex statistical distribution. Our results showed a tendency for the standard errors to increase with eelgrass depth limit at lower depths and with a flattening out at larger depths. Therefore, we used either a Gaussian or spherical model to fit the relationship between standard errors and expected max depth limit. PROC MIXED and PROC MODEL in SAS 9.2 (Cary, NC) were used for conducting all analyses.

The established relationships between standard errors and expected depth limit were used to calculate the uncertainty of an estimated mean max depth limit based on n_A years, n_B divers, n_C transects and n replicates for each transect monitored in a given year by a given diver. Assuming a balanced design, in practice most likely with rotation of divers across years and transects, the mean depth limit can be estimated from the observations y_{ijkl} as:

$$
\begin{aligned}
\overline{Y} &= \sum_{i=1}^{n_A}\sum_{j=1}^{n_B}\sum_{k=1}^{n_C}\sum_{l=1}^{n} y_{ijkl} \\
&= \sum_{i=1}^{n_A}\sum_{j=1}^{n_B}\sum_{k=1}^{n_C}\sum_{l=1}^{n} \mu + A_i + B_j + C_k + e_{ijkl} \qquad \text{(Eq. 2)} \\
&= \mu + \frac{\sum A_i}{n_A} + \frac{\sum B_j}{n_B} + \frac{\sum C_k}{n_C} + \frac{\sum\sum\sum\sum e_{ijkl}}{n_A \times n_B \times n_C \times n}
\end{aligned}
$$

having a variance of:

$$
V\left[\overline{Y}(depth)\right] = \frac{\sigma_A^2(depth)}{n_A} + \frac{\sigma_B^2(depth)}{n_B} + \frac{\sigma_C^2(depth)}{n_C} + \frac{\sigma^2(depth)}{n_A \times n_B \times n_C \times n} \qquad \text{(Eq. 3)}
$$

where variances for the different uncertainties are formulated as functions of the expected max depth limit, using the established relationships described above. In the Water Framework Directive observations are used to characterise a planning period of 6 years. In that case, the variance contribution from different years should be relative to the variation among the 6 years and not related to the entire population of years, i.e. if all years within the 6-year period are monitored then the distribution of the interannual variation is essentially known. This has repercussion for the variance of the estimated max depth limit, which in this case changes to:

$$V\left[\overline{Y}(depth)\right] = \frac{\sigma_A^2(depth) \times (1 - n_A/6)}{n_A} + \frac{\sigma_B^2(depth)}{n_B} + \frac{\sigma_C^2(depth)}{n_C} + \frac{\sigma^2(depth)}{n_A \times n_B \times n_C \times n} \quad \text{(Eq. 4)}$$

showing that the variance from the interannual variations becomes zero when all years are sampled. This variance equation (Eq. 4) was used to calculate the uncertainty of the mean max depth limit for various combinations of number of monitored years, transects, and replicates as well as number of divers used for the monitoring.

Optimising monitoring efforts

Increasing the number of observations, whether it is number of years, divers, transects or replicates, will inevitably reduce the uncertainty of the estimated mean depth limit, however, changing these numbers for monitoring effort will affect the uncertainty differently. To illustrate the use of variance components in designing a monitoring program we will minimise the uncertainty under monitoring constraints in the form of maximum man hours to be allocated for eelgrass monitoring. The time required for the different activities has been estimated by experienced people in eelgrass monitoring (M. B. Rasmussen, pers. comm.). The conduct of field campaign to monitor eelgrass requires 3 people: a diver, a line holder, and a ship master. The planning of the field campaign is assumed to take 8 hours in total per year of monitoring. Transport for the 3 people including boat time to the area of investigation and back again, is assumed to take 4 hours per person. Monitoring of a single transect commonly takes 0.5 hour and an additional 3 minutes (~0.05 hour) for each replicate. Transport from one transect to the next is approximately 0.5 hour. If there is more than one diver on the field campaign, the divers are assumed to monitor parallel transects from the boat simultaneously and thus not adding to the total time required for monitoring the transect. The time required for eelgrass monitoring in a given water body can be calculated as

$$\text{Time (hours)} = n_A \times (8 + (2 + n_B) \times (4 + n_C \times (0.5 + (n - 1) \times 0.05) + (n_C - 1) \times 0.5)) \qquad \text{(Eq. 5)}$$

where the number of years (n_A) of monitoring is multiplied by 8 hours of planning and the time required to conduct the field campaign in a given year. The number of people on the boat is two plus the number of divers (n_B), using 4 hours as a base cost for conducting the field campaign plus a cost for the number of transects (n_C) and the transport between transects.

The uncertainty of the estimated eelgrass mean depth limit (Eq. 4) for a given monitored water body was estimated for various combinations of number of years (i=1 to 6), number of divers (j=1 to 3), number of transects (k=1 to 8), and number of replicates (l=1 to 10). The combinations that resulted in the lowest variance of the eelgrass mean depth limit (Eq. 4) under time constraints of 100 and 200 hours were chosen as the optimal design for the monitoring program.

RESULTS

Sources of variation in general

The maximum depth limit for all transects varied between 0.2 m and 12.5 m. The maximum depth limit differed significantly between sites (mixed model $F_{6, 7278}=26.4$, $p<0.0001$). Variation between transects was larger than variation between year, diver, and replicates (Figure 3, Table 2).

The standard error increase with the estimated maximum depth limit

Normally, variances are assumed constant across their range, but for transect, diver, year and replicate the variance overall increased with the estimated maximum depth limit (Figure 4). For all variables the standard error initially increased with the estimated maximum depth limit and then levelled off. We fitted either a Gausian or a Spherical function to parameterize the relation between the uncertainty and the depth limit, and selected the function that resulted in the best fit to the data (Table 3). A Gaussian function gave the best fit for year ($R^2=0.11$), where the uncertainty increased with the depth limit until 2.73 m and then levelled off at a standard error of 0.48 m (Table 3, Figure 4a).

For the uncertainty that can be associated with diver a spherical function gave the best fit ($R^2=0.23$) to describe the relation between uncertainty and depth limit. For diver the range estimate suggested that the uncertainty of the maximum depth estimate (i.e. standard error) increased until 7.18 m maximum depth and then stabilized around a standard error of 0.75 m (Table 3, Figure 4b). The variation between transects increased until 1.82 m and stabilized at a standard error of 0.77. Regarding the variation between years, the range suggested that the standard error increased until reaching a maximum depth limit of 2.32 m, after which it stabilized at a standard error of 0.47 (Table 3, Figure 4c). The replicates were best approximated by a spherical function ($R^2=0.21$). The range estimate for replicate variation indicated that the standard error increased with the maximum depth until 4.65 m and subsequently stabilized at standard error of 0.54 m (Figure 4d).

We used equation 3 to calculate the total depth specific variance (Figure 5). The total variance on the depth limit estimation increased with the depth limit until 7.26 m.

Minimizing uncertainty in a survey design

The functions above (Table 4) can estimate the variance at any depth limit for the 3 random factors and the replicates and thereby estimate the total variance. Obviously larger sampling effort in terms of number of years, divers, transects, and replicates will reduce the uncertainty of the estimates of a depth limit at a water body. However, in reality the available resources for surveys often constrain the sampling efforts. The challenge is to optimize the sampling effort to attain a minimal uncertainty in estimates of eelgrass depth limits at given limited resources. We used eq 5 to determine possible combinations of numbers of year, diver, transects, and replicates within 100 h or 200 h available for survey of a area. For all possible combinations of numbers of year, diver, transect and replicates we calculated the depth specific variance using eq 4.

For a maximum of 100 h spent on a survey the minimum variance on an estimate of the depth limit was 0.451 for shallow depth limits (3 m) and 0.607 for deep depth limit (6 m). The minimal variance could be achieved by the combination of 2 years, 3 divers, and 4 transects with 5 replicates. The 20 combinations that resulted in lowest variances (ranging from 0.451 to 0.510 for shallow depth limits and between 0.608 and 0.682 for deep depth limits) all monitored over 2 years, used 2 or 3 divers, and between 3 and 7 transects with the majority being 5 transects for shallow depth limits and 3 transects for deep depth limits. Replicates for theses combinations varied between 1 and 10 replicates. For a maximum of 200 hours spent on a survey the minimum variance for the depth limit estimate was 0.303 for shallow and 0.434 for deep depth limits, which could be achieved by a combination of 3 years, 3 divers, 8 transects and 1 replicate. The 20 combinations with the lowest variances ranged from 0.303 to 0.353 for shallow depth limits and 0.434 to

0.486 for deep depth limits. The majority of these 20 combinations used 3 years and 2 would use 4 years, all used 3 divers, transects varied between 4 and 8 and replicates varied between 1 and 10. In reality most surveys are conducted with only one diver on the boat. For a 100 h surveys the best combination with 1 diver gave a variance of 0.654 for shallow depth limits and 1.011 for deep depth limits (Figure 6a) and consisted of 2 years, 8 transects, and 7 replicates. For a 200 hour survey the minimum variance using only 1 diver was 0.560 for shallow depth limits and 0.889 for deep depth limits (Figure 6b), which consisted of a combination of 4 years, one diver, 8 transects, and 7 replicates.

DISCUSSION

All the variance components were found to be important for the uncertainty of the depth limit estimates of eelgrass. Yet the sources of the variation likely differ between variance components. Variation in depth limits between years likely depends primarily on year to year variation in light availability, the main factor governing depth limits (e.g. Duarte et al. 2007). Changes in turbidity, for instance due to algal blooms, would alter the light conditions and affect the depth limit, because the photosynthesis gain and respiration loss just balance here. Interannual variation in factors beyond light may also affect the suitability of the habitat for eelgrass growth (Koch et al. 2001) and induce variations in depth limits. The extent and duration of hypoxia or anoxia, which may severely hamper eelgrass (Pulido & Borum 2010) is a probable source of variation. Blue mussels (*Mytilus edulis*), which inhabit some of the same depths as eelgrass, are fished for consumption. The current fishery procedure involves dredging of the sea bottom in Skive Fjord and other Limfjord basins in cycles of a few years, and may physically remove the eelgrass. Although mussel dredging is only allowed to a certain minimum depth, located deeper than eelgrass, it cannot be excluded that it occasionally affects eelgrass depth limits. Interannual variation in physical factors such as storms may also affect especially shallow eelgrass populations either directly (Fonseca et al. 2002) or indirectly through modifications of the substrate (Koch et al. 2001; Krause et al 2011a). Furthermore, the exact location of transects may vary between years, thereby causing variation in depth limits, especially if the eelgrass distribution is patchy.

Small deviations in individual divers practice during estimation of the depth limit and the accuracy with which they can correct for fluctuation in water level during a day of surveys is the likely main cause of diver variation. Furthermore, when divers swim the zigzag across the depth limit they will invariably cross the depth limits at different locations, which could lead to different estimates especially if the distribution of eelgrass is patchy.

Large-scale spatial variation between transects in a water body is most probably caused by some of the same factors causing interannual variation in depth limits, e.g. difference in light climate, oxygen conditions and substrate conditions e.g. due to difference in eutrophication pressures and wind exposure within the water body.

The replicates within a transect reflect small scale spatial variation which is strongly dependent on the patchiness of eelgrass. Replicates representing the same large eelgrass patch are likely less variable than those reflecting a patchy eelgrass distribution.

Differences in patchiness may, thus, explain some of the difference in variation between transects and replicates in Figure 2a and 2b.

Why does the uncertainty increase with the estimated max depth limit?

The increase in uncertainty of all variance components with increased maximum depth limit (Figure 4 & 5) suggests that deeper depth limits were more variable on temporal as well as on spatial scales and also more difficult for the divers to determine. The reason could be that the zone of light levels supporting eelgrass at the depth limit is more extended in clear than in turbid waters. Large depth limits reflect relatively clear water and hence less light attenuation, which means that the area exposed to for instance 5 % surplus light (photosynthesis relative to respiration) and 0 % surplus light (max depth limit) would be larger for large depth limits. Given this scenario the observed depth limit at large depth could be expected to fluctuate more than at shallow depth limits.

Also, the range of possible depth limits is larger in clear water with deep depth limits than in turbid waters with shallow depth limits and thereby allows a large inherent variation. The shore line thus sets a lower boundary for the depth limit estimates which limits the variation in shallow depth limits.

The uncertainty for divers continued to increase until 7.3 m before it leveled off. This may reflect an increased difficulty in estimating the depth limit at deeper depths and, again, an increased range of possible depth limits. Despite a well-defined start point for the transects, the diver may drift to either side of the transect, such drift may accumulate in long transects resulting in larger variation in long transects (i.e. at larger max depth limit). However, the relative variation in depth limits actually declines with larger max depth limit, as the variation makes up a smaller proportion of the larger than the smaller estimated depth limits. This decrease in relative variation also means that it should be easier to detect relative changes in deeper depth limits than in shallower depth limits. The higher relative variation in shallow depth limits may be due to larger effect of physical exposure, causing larger variability in eelgrass distribution and abundance towards the shore (Krause-Jensen et al 2000, Fonseca et al. 2002). For the variance components 'year' and 'transect' the uncertainty leveled off already around 2 m, which could be the depths where physical factors such as wind exposure have smaller effect and the variation between transects and years therefore becomes stable.

Parameterization of uncertainty

In this study we parameterized the uncertainty for the variables associated with the survey design. Such quantification of the uncertainty is important for evaluating the accuracy of ecological status class assessment in surveys to test for compliance with the water framework directive (Carstensen 2007).

The formulas that we here present are generally applicable to marine monitoring programs and should be taken into account when designing a survey. In our study it was possible to estimate the parameters empirically as we had access to a large database where no time series trend existed in the eelgrass depth limit (Hansen & Petersen 2011; Carstensen et al. 2012). Our eelgrass data set had special characteristics as the uncertainty for all variables increased with the depth limit. At shallow depths the uncertainty increased with the depth limit i.e. log normally distributed, this can usually be transformed to fit normality with a log transformation, whereas the uncertainty at larger depth limits remained unaffected by increases in the depth limit and thus follow a normal distribution.

Implications for survey designs

We can use the parameterization of the variance component to estimate the variance for different survey designs. The results of our case studies give clear recommendations for design of surveys that can reduce the uncertainty relative to the current survey designs. In particular using 2 or 3 divers simultaneously instead of just 1 diver can apparently reduce the variance with up to 40 %.

Despite that a six year period was available for the survey the variance on the depth limit estimation would be smallest for only 2 or 3 years of survey for both time-limited scenarios, and transects varied between 4 and 8 transects per water body. It is, however, interesting that the residuals seemed to have little effect on the uncertainty.

We admit that our approach to calculation of a time budget is simplified, but it illustrates an important point, which is that the diver can have large effects on the uncertainty and survey would need to limit the uncertainty with which they contribute. Similar effect of surveyor has been documented for benthic invertebrates (Benedetti-Cecchi et al 1996). The use of multiple divers surveying a transect will undoubtedly improve the accuracy of the estimate of the depth limit for a water body. However, given the individual differences between divers it would be easier to detect a change in a water body if transects were surveyed by the same diver year after year. Some regional surveys have employed the strategy of using the same diver to survey specific transects. Such strategy, however, is vulnerable to changes in diver, which would disrupt the reliability of the whole time series. Unless one is sure to be able to use the same diver for many years such a sampling strategy would not benefit the accuracy of estimates of eelgrass depth limit over longer time span.

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TABLES

Table 1. Overview of the data set for the monitored estuaries.

Water body	Area (km^2)	Min. depth (m)	Max depth (m)	#areas	# transects	# years	# divers	# observations
Aabenraa Fjord	32.1	2.8	12.3	3	19	8	4	679
Als Fjord	35.1	1.0	6.5	1	3	6	1	151
Augustenborg Fjord	15.0	1.3	3.9	1	6	6	2	230
Dybsø Fjord	17	0.6	2.0	1	5	3	4	63
Ebeltoft Vig	85.4	5.8	7.7	1	5	2	2	71
Endelave	61.8	3.5	6.9	1	4	4	2	64
Fakse Bugt	673.1	4.3	9.6	1	7	1	2	35
Flensborg Fjord	281.8	0.7	9.5	2	15	8	2	748
Genner Bugt	4.4	1.7	5.4	1	3	5	1	84
Guldborg Sund	81.7	4.9	8.0	1	1	4	2	21
Haderslev Fjord	2.6	0.8	5.5	1	4	5	2	66
Helnæs Bugt	66.2	3.3	5.6	1	1	5	2	39
Hevring Bugt	544.4	0.8	5.2	1	7	4	3	93
Hjelm Bugt	249.1	7.3	8.6	1	3	2	2	4
Holbæk Fjord	14.1	2.5	4.7	1	3	5	5	58
Horsens Fjord	78.2	0.5	5.7	3	13	7	4	172
Inder bredning	62.2	2.0	6.7	1	5	6	6	77
Kalundborg Fjord	55.4	1.6	8.5	2	11	6	4	140
Karrebæk Fjord	16.0	2.5	9.5	1	5	5	4	88
Kattegat N	645.1	2.2	6.2	1	2	3	1	36
Knebel Vig	7.4	2.0	5.0	1	3	1	1	25
Kolding Fjord	8.9	0.6	4.8	2	9	7	5	95
Korsør Nor	7.8	2.0	2.2	1	1	1	1	4
Køge Bugt	364.2	5.1	8.8	1	6	7	4	185
Lammefjord	20.6	1.6	1.7	1	1	1	1	2
Langerak	33.9	1.1	3.2	1	4	9	5	140
Lillebælt Nord	109.7	0.7	7.0	1	5	5	3	54
Lillebælt Syd	187.6	0.2	12.5	1	4	8	2	156
Limfjorden	1517.0	0.6	3.8	3	24	9	9	920
Lovns Bredning & Skive Fjord	105.0	0.5	3.8	1	7	8	7	293
Mariager Fjord	45.6	0.5	2.7	2	10	5	3	125
Nakkebølle Fjord	6.7	3.6	6.7	1	1	2	1	20
Nibe Gjøl Bredning	135.7	0.3	3.4	1	9	8	5	396
Nissum Bredning	239.0	1.0	3.6	1	2	6	7	79
Nissum Fjord	64.3	1.3	1.4	1	1	1	1	7
Nivå Bugt	51.7	4.6	9.1	1	7	5	4	113
Odense Fjord	61.8	1.2	6.6	3	17	3	2	251
Præstø Fjord	22.0	3.4	5.6	1	6	5	4	98
Ringkøbing Fjord	283.4	0.9	1.5	1	3	8	4	85
Roskilde Fjord	124.8	0.2	8.7	2	19	6	6	265

Sejerø Bugt	755.8	1.3	9.0	1	9	7	4	149
Smålands farvandet	1559.1	5.1	8.0	1	8	5	4	108
Storebælt	4522.2	6.0	6.1	1	1	1	1	4
Sydfynske Øhav	468.4	2.0	7.5	1	16	7	1	186
Tempelkrog	3.6	1.2	4.2	1	2	6	5	35
Vejle Fjord	107.8	0.8	5.6	2	13	8	6	242
Øresund	1356.5	3.4	9.4	1	11	7	4	331
Yderbredning	253.3	4.4	9.8	1	9	7	7	149
Århus Bugt	315.9	2.3	7.6	3	10	5	6	301

Table 2

Variance parameter estimates ± SE, p-values nad number of observations (n) for the random effects in the model on the full data set.

Variance component	Parameter		Combined model			Area-specific values		
			Estimate	p-value	N	Min.	Mean	Max.
Year	σ^2_α	α_i	0.212	0.031	53	0.028	0.374	1.775
Diver	σ^2_β	β_j	0.165	0.009	37	0.101	0.501	1.508
Transect	σ^2_γ	γ_l	0.769	<0.0001	52	0.044	0.672	2.049
Replicate	σ^2_{δ}.	$\delta_{l(k)}$	0.366	<0.0001	58	0.087	0.461	1.053

Table 3.

Model fits for the standard error of the four random components. Range indicated when the sill (Threshold value) was reached, which is when the standard error ceased to increase with the estimated maximum depth limit. Sill estimated the threshold value for the function. Nugget indicated intercept with the y-axis. We used range=4 and sill=0.5 as start values for the estimation procedure.

Variance component	Function	R^2	Parameter estimates		
			Range (m)	Sill (m)	Nugget (m)
Year	Gaussian	0.11	2.73	0.39	0.09
Diver	Spheric	0.23	7.26	0.75	0
Transect	Gaussian	0.13	1.82	0.77	0
Replicate	Spheric	0.21	4.65	0.55	0

FIGURE LEGENDS

Figure 1. A. Water bodies in Denmark where eelgrass depth limits are surveyed. B. Example of distribution of Transects in Vejle Fjord. C. The solid line marks the transects and the dotted line illustrates the diver zigzag route crossing the max depth limit.

Figure 2. Replicate observations of the depth limit for three transects at each site between 2001 and 2009. The replicate observations of the depth limit of eelgrass within a transect within a year vary considerably. Observation from the three transects in each area are marked with +, -, X. Transects were located in Vejle fjord (A), and Aabenraa fjord (B).

Figure 3. The estimates for the uncertainty (SE) contribution of year, diver, transect and replicate on maximum depth limit.

Figure 4. The standard error as a function of the estimated maximum depth limit for eelgrass for Transect; Diver; Year; and Replicate. Lines show the fitted spherical or Gaussian functions.

Figure 5. Total variance as a function of depth calculated by equation 4.

Figure 6. Minimum variance at different depths for one diver(black), two divers (white) and 3 divers (hatched) for A) 100 h survey and B) 200 h survey. The variance is calculated by eq 3.

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Ecological status of seagrass ecosystems: An uncertainty analysis of the meadow classification based on the *Posidonia oceanica* multivariate index (POMI)

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A B S T R A C T

Quantifying the uncertainty associated with monitoring protocols is essential to prevent the misclassification of ecological status and to improve sampling design. We assessed the *Posidonia oceanica* multivariate index (POMI) bio-monitoring program for its robustness in classifying the ecological status of coastal waters within the Water Framework Directive. We used a 7-year data set covering 30 sites along 500 km of the Catalonian coastline to examine which version of POMI (14 or 9 metrics) maximises precision in classifying the ecological status of meadows. Five factors (zones within a site, sites within a water body, depth, years and surveyors) that potentially generate classification uncertainty were examined in detail. Of these, depth was a major source of uncertainty, while all the remaining spatial and temporal factors displayed low variability. POMI 9 matched POMI 14 in all factors, and could effectively replace it in future monitoring programs.

1. Introduction

Degradation in water quality resulting from anthropogenic disturbance poses a large threat to both freshwater and marine ecosystems. In response to this, the European Union has developed the Water Framework Directive (WFD), a trans-national strategy aimed at maintaining and, where necessary, recovering the water quality of aquatic ecosystems across European member states. The main objective of the WFD is to achieve, at least, a 'good ecological status' for all water bodies across Europe by 2015. The concept of 'ecological status', as defined by the WFD, is the quality of the structure and functioning of aquatic ecosystems associated with surface waters. The ecological status is determined by monitoring and assessing biological indicators relevant to the water body in question that are integrated into an index with the aim to detect temporal and spatial changes in water bodies. Under the WFD, the values of this index, typically from zero to one, are used to classify each water body into one of five classes, from bad to high. However, a reliable biotic index should not only be able to detect change, but also know that the change it is detecting is meaningful and not an artefact of the methodology, sampling design or execution. Uncertainty analyses are a useful tool to identify the factors which contribute to the potential misclassification of the ecological status class of water bodies (Clarke and Hering, 2006; Staniszewski et al., 2006), which could result in considerable social, economic and ecological losses to the region.

Within coastal waters, four biological quality elements (BQEs) have been identified: phytoplankton, macroalgae, angiosperms and benthic invertebrate fauna. These BQEs are sensitive to anthropogenic disturbances and, therefore, are potential indicators of the ecological status within the water body. Seagrass ecosystems, and in particular for the Mediterranean *Posidonia oceanica* meadows, make an ideal BQE for monitoring ecological status because of their high sensitivity to disturbance (Delgado et al., 1999; Francour et al., 1999; Ruiz et al., 2001; Ruiz and Romero, 2003), wide distribution (Procaccini et al., 2003) and well understood biology and ecology (Romero et al., 2007). In regions of Spain and Croatia, the POMI index (*P. oceanica* multivariate index; Romero et al., 2007) is used to monitor, evaluate and classify the ecological status of coastal water bodies. POMI incorporates 14 metrics from physiological, individual, population and community levels, making it sensitive to both lethal and sub lethal stressors impacting multiple levels of organisation (Martinez-Crego et al., 2008). These metrics are integrated, using principal components analysis (PCA), into a single value from which an EQR (ecological quality ratio, from zero to one) is obtained (Romero et al., 2007).

Posidonia oceanica meadows are dynamic systems, with a constant flux of intrinsic and extrinsic factors influencing their structure and function over multiple spatial and temporal scales. Such dynamics have the potential to obfuscate meaningful trends in ecological status predictions, if the sources of variability are not

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recognised and where possible, reduced. When designing a monitoring program, decisions must be taken on how to assign the total sampling effort within the allocated time and budget constraints. The number and distribution of replicates should be taken with the objective of maximising precision in mind. For example, seagrass meadows usually display horizontal heterogeneity (e.g. zones of different density, structure, depth, etc.) within a meadow, and multiple, spatially independent meadows may exist within a water body. Whilst it is expected that sites within a water body reflect similar ecological statuses, a certain level of variability among sites is inevitable. Establishing a measure of the variability among meadows within a water body enables an estimation of the likely precision of ecological status estimates from water bodies where only a single site is present. Uncertainty analyses enable us to visualise how our sampling precision is influenced by alternative sampling designs. However, despite its fundamental importance, there is little information on how these different scales and factors can be used to minimise uncertainty in coastal waters, particularly in seagrass meadows.

The current study analyses uncertainty in classifying the ecological status of *P. oceanica* ecosystems, based on the POMI biomonitoring program, with two key areas of focus. The first is to compare POMI (hereafter referred to as POMI14) to a condensed 9 metric version (POMI 9) to assess how uncertainty estimates are affected by the reduction in the number of metrics. The second area of focus will be to determine which sources of variability (factors) associated with the sampling design of the POMI monitoring program most greatly influence ecological status classifications of *P. oceanica* meadows. The analyses will be based on a seven year data series that includes over 30 meadows and the factors analysed will include spatial scales of sampling (variability among zones within a meadow, among meadows within a water body, variability among depths), the temporal scale of sampling (variability among years) and the human-associated source of error (variability between surveyors). These five factors represent some the key sources of variability associated with the design and implementation of a regional scale bio-monitoring program, and highlight how certain elements of a sampling design can influence the precision and the probability of misclassifying the ecological status of coastal water bodies.

2. Materials and methods

2.1. Site selection and sampling

Bio-monitoring data from *P. oceanica* meadows were collected from 30 sites spanning 500 km of the Catalonian coastline (Spain). Sampling took place in autumn (September–October) throughout the years 2002–2009, with the exception of 2004, when sampling took place during early summer (June) and was, therefore, excluded from the analysis. Sites used in the study covered a wide environmental gradient and a broad ecological status range, representing 17 coastal water bodies along the Catalonian coastline (Fig. 1). The spatial extent and division of coastal water body boundaries are classified by the WFD according to the water quality and anthropogenic pressures to which the coastline is exposed, independent of the number, or status, of seagrass meadows within the area *per se*. The number of sites within a water body ranges from 1 to 7 along the Catalonian coastline and sites within a water body can be up to 50 km apart. Within each site, 14 biological metrics were determined for POMI. Among them, 5 metrics are representative of the physiological level (phosphorous, nitrogen and sucrose content and δ^{15}N and δ^{34}S isotopic ratios in rhizomes); 2 of the individual level (percentage of leaves with necrosis and shoot leaf surface area); 3 of the population level (meadow cover,

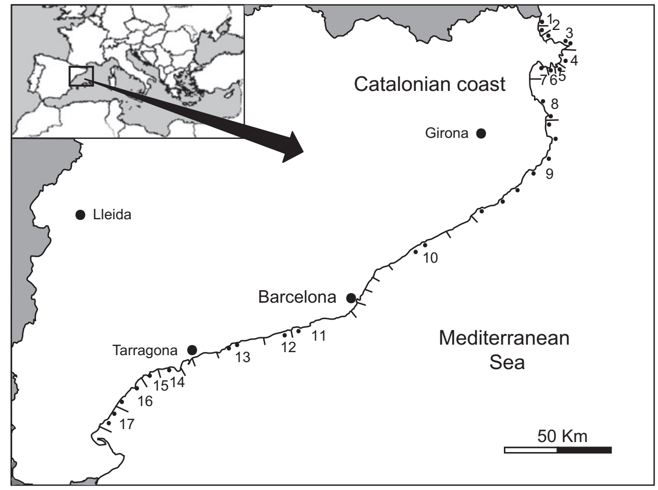

Fig. 1. Map of the location of the 30 sampling sites (small black dots) and 17 water bodies along the Catalonian coast, where the index was applied.

shoot density and percent of plagiotrophic rhizomes); 1 of the community level (nitrogen content in the epiphytes) and 3 are pollution indicators (copper, lead and zinc concentration in rhizomes). POMI 9 was based on the same group of metrics as POMI 14, excluding, however, phosphorous and nitrogen content in rhizomes, copper and zinc concentrations in rhizomes and percent of plagiotrophic rhizomes. POMI 9 attempts to minimise the redundancy of metrics within the different organisational levels present in POMI 14, whilst retaining a high sensitivity to change.

2.2. Classification of the ecological status

To determine the ecological status class of *P. oceanica* meadows, each meadow (site) was scored on a scale from 0 (worst conditions, BQE severely damaged or missing) to 1 (optimal reference conditions of BQE). Scores were obtained as EQRs (ecological quality ratio), which is the ratio between the POMI scores from a given site and the scores for the reference conditions. To do this (1) reference conditions for each POMI metric were identified and (2) POMI metrics were combined onto a single scale (see below). In the current study, the reference conditions were taken to be the mean value of the best three scores for each respective metric, following Romero et al. (2007). Similarly, the worst condition of each metric was recorded as the mean value of the three worst scores for that particular metric. Note that optimal conditions reflected either high or low values depending on the metric in question (e.g. high shoot density is optimal whilst low lead concentration is optimal). Metrics for POMI 9 and POMI 14 were each combined onto a single scale using principal component analyses (PCA). The resultant score of each sampling site, on the first axis of the PCA was then used to calculate its EQR and determine its ecological status class. EQRs were calculated for each site using the equation:

$$EQR'_x = (CI_x - CI_{worst})/(CI_{optimal} - CI_{worst}) \quad (1)$$

where EQR′ is the ecological quality ratio of site x, CI_x, CI_{worst} and $CI_{optimal}$ are the scores of site x, the worst reference site and the optimal reference site on the first component respectively.

The ecological status of each site was classified into one of five classes from ‘high’ to ‘bad’, set within the 0–1 EQR scale. Because of its sensitivity to anthropogenic disturbance, *P. oceanica* does not survive in extremely bad conditions, therefore, no matter how degraded the meadow, all sites used in the current study were considered better than ‘bad’ (because *P. oceanica* was present). The EQR for the “bad” class was set to the range 0–0.1, and the upper four status class boundaries were determined by dividing the remaining EQR scale (0.1–1) into four equal categories, following Romero et al. (2007). The EQR of each site was, therefore, classified into one of the upper four status classes using the equation:

$$\mathrm{EQR}_x = (\mathrm{EQR}' + 0.11)/(1 + 0.10) \quad (2)$$

2.3. Variability within factors

The five factors examined in the current study that potentially contribute to the uncertainty of the EQR estimations of coastal water bodies include variability among (1) zones within a site (meadow), (2) sites within a water body, (3) depths, (4) years and (5) surveyors. In total, 30 sites were sampled from 17 water bodies. Within each site three zones were sub-sampled. In the annual monitoring program, metric values are averaged among zones to provide robust estimates within each site. For the purpose of the study, however, inter-zonal variation was estimated to establish estimates of small scale meadow heterogeneity. Inter-annual variation in mean EQR scores was estimated among the years 2002–2009, with the exception of 2004 (to avoid potential inter-seasonal variation). Variation in depth ranged between 10 and 17 m (14.63 ± 0.28 m, mean ± SE) among stations, throughout the standard annual POMI monitoring program. In addition to this, however, during 2002 POMI scores were estimated from 5 m zones in four sites. The 5 m POMI scores were included to observe the variability across a broad depth range. Variation between 'surveyors' was estimated by comparing POMI values between two trained, skilled surveyors from 30 sites during 2008 and 16 sites during 2009. Of the POMI metrics, only estimations of meadow cover were subject to variation between surveyors. To account for that variability each replicate quadrat was sampled by both divers (see Martinez-Crego et al. (2008) for methodology). POMI 14 and POMI 9 were calculated for each surveyor, by using their respective meadow cover estimates in the PCA, whilst maintaining the same values for the remaining metrics.

The total variance and variance components associated with each factor were estimated for POMI 14 and POMI 9 using a linear mixed effects model in the lme4 package of R (Bates, 2005, 2007, Version 2.10.1, R_Development_Core_Team 2009). The spatial factors were treated as random nested intercepts, with zones nested within sites and sites nested within water bodies. Note that the variability among water bodies, whilst important in the analysis of variance components is not presented in the results of this study because the WFD is interested in the status of BQE's up to the spatial scale of water bodies, not among water bodies, which by definition should differ in their ecological status. The remaining three factors, 'year', 'depth' and 'surveyor' were each treated as random crossed intercepts. Variance components were determined by calculating the proportion of the total variance (σ^2_{T}) explained by each individual factor. Total variance in EQR for each POMI was given by:

$$\sigma^2_{\mathrm{T}} = \sigma^2_{\mathrm{Z}} + \sigma^2_{\mathrm{Si}} + \sigma^2_{\mathrm{WB}} + \sigma^2_{\mathrm{Y}} + \sigma^2_{\mathrm{Su}} + \sigma^2_{\mathrm{D}} + \sigma^2_{\mathrm{R}} \quad (3)$$

where σ^2 = variance due to differences in mean EQR values among zones (σ^2_{Z}) within a site, among sites (σ^2_{Si}) within a water body, among water bodies (σ^2_{WB}), among years (σ^2_{Y}), between surveyors (σ^2_{Su}), among depths (σ^2_{D}), and the residual variance (σ^2_{R}) in mean EQR values not explained by the model. The proportion of total variance (P_{samp}) explained by each factor was given by the equation, following Clarke et al. (2006):

$$P_{\mathrm{samp}} = 100\sigma^2_x/\sigma^2_{\mathrm{T}} \quad (4)$$

Finally, the total variance estimates associated with the classification within individual water bodies monitored by POMI was determined for two different sampling designs by summing the variances of the contributing factors. Ecological status predictions based on the current monitoring program along the Catalonian coastline (controlled design, σ^2_{CD}) is subject to uncertainty in classification based on the variability among meadow depths (10–17 m) and among sites within a water body

$$\sigma^2_{\mathrm{CD}} = \sigma^2_{\mathrm{Si}} + \sigma^2_{D^*} \quad (5)$$

whereby $\sigma^2_{\mathrm{D}^*}$ is the variance due to differences in mean EQR value among the different depths from sites used in the annual monitoring program (10–17 m). Inter-zonal, inter-annual and inter-surveyor variability was incorporated into this design by averaging metric values among multiple replicate zones, multiple years and with multiple surveyors to provide robust estimates of the ecological status class of each water body and, therefore, were not considered in total variance estimations. Total variance estimates of a second, hypothetical 'uncontrolled design' (σ^2_{UD}) where variability among zones, years, surveyors and a broader depth range (5–15 m) are not controlled for, was simulated by the equation:

$$\sigma^2_{\mathrm{UD}} = \sigma^2_{\mathrm{Z}} + \sigma^2_{\mathrm{Si}} + \sigma^2_{\mathrm{Y}} + \sigma^2_{\mathrm{Su}} + \sigma^2_{\mathrm{D}} \quad (6)$$

This equation demonstrates the variability that would be implicit in a sampling design where depth was not controlled among sites, or where only a single zone within a site was monitored. The resultant EQR score for a water body, under such a sampling design, would not account for the natural variability therein, and would be exposed to a higher probability of misclassifying the ecological status of the water body. All data adequately satisfied the assumption of homogeneity of variance based on plots of the residuals against the fitted EQR values, therefore, no transformation of the data took place.

2.4. Analysis of uncertainty

Having calculated the variability within each factor and index, the uncertainty in ecological status classification was estimated using STARBUGS (STAR bioassessment uncertainty guidance software, Clarke, 2004). STARBUGS helps determine whether an observed ecological status classification is indeed the most probable classification for a particular site, given the inherent sources of variability. STARBUGS sums the observed value for a given site with a random standard normal deviate, of the known SD, with a mean of zero (Clarke and Hering, 2006). It repeats this simulation 10^4 times to produce a frequency distribution of possible EQR values for the particular site or water body: The simulated EQR values are grouped into their corresponding status classes, from which the probability of misclassifying the original observed value can be determined. Because the current study was interested in the uncertainty in classification generated by particular factors (rather than the probability of misclassifying individual sites), the simulation was repeated for the full range of possible observed EQR values (0–1).

3. Results

The factors 'surveyor', 'year', 'site' and 'zone' displayed relatively low levels of uncertainty in the ecological status classification of water bodies based on both POMI 9 and POMI14, whilst 'depth' resulted in relatively high probability of misclassification (Fig. 2). The probability of misclassification, for all five factors, peaks when a site's observed EQR score is very close to the boundary between two status classes, reaching values of 50%. Similarly, when the observed EQR falls in the middle of a status class the probability of misclassification declines to as low as <0.001%, depending on the variability in EQR scores associated to the factor

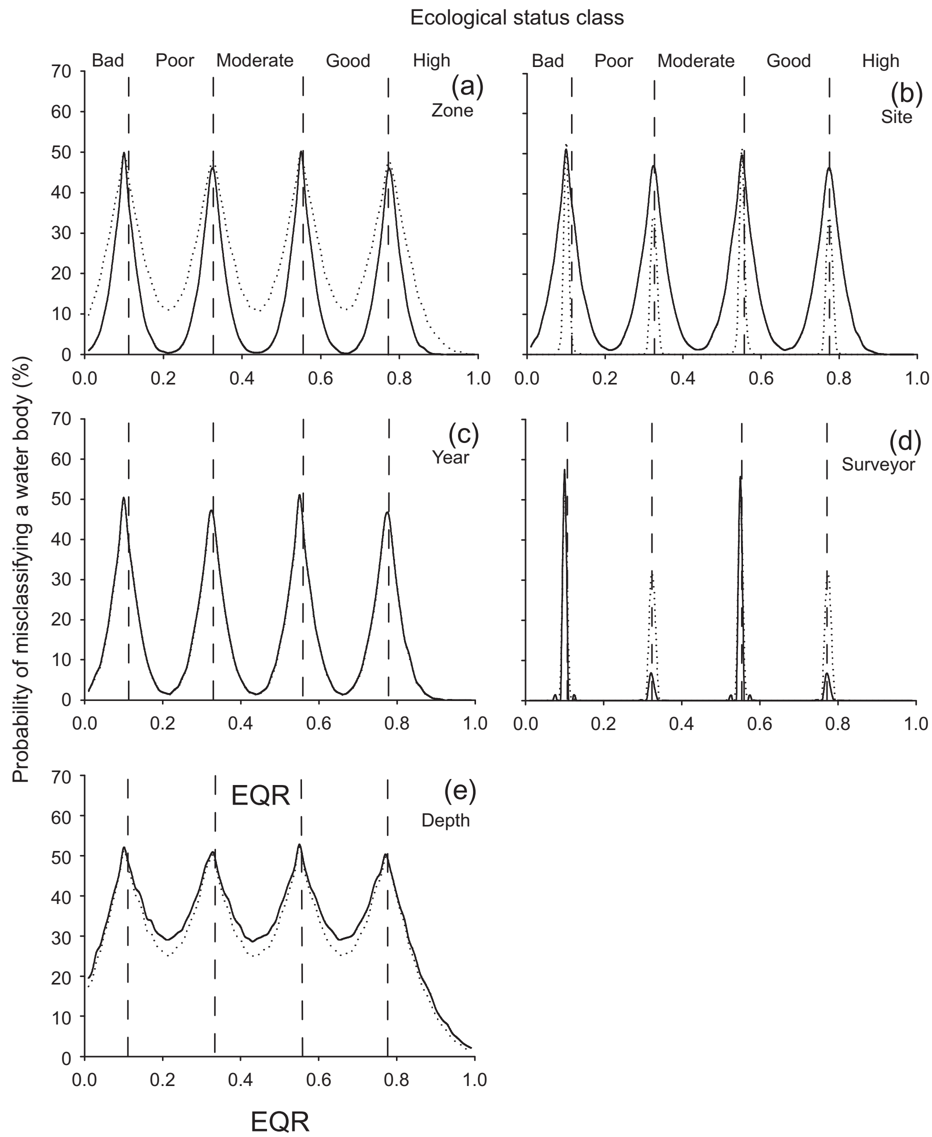

Fig. 2. Probability of misclassifying the ecological status class given the variability among mean EQR values calculated by POMI; (a) among zones within sites, (b) among sites within water bodies (c) among years, (d) among surveyors and (e) among depths. Bold and dotted lines represent POMI 14 and POMI 9, respectively. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 to 0.099, poor = 0.1 to 0.324, moderate = 0.325 to 0.54, good = 0.55 to 0.774 and high = 0.775 to 1.

in question. The higher the variability, the higher its probability of misclassification even in the centre of the status class ranges.

Among the nested factors, variability among zones and among sites both demonstrated relatively low variability for both POMI 14 and POMI 9 (Table 1). This corresponded with ⩽10% probability of misclassification in the centre of the status class range, based on the variability among zones (Fig. 2a), and <2% probability of misclassification based on the variability among sites, for both POMI 14 and POMI 9 (Fig. 2b). In contrast, the highest levels of variability were observed in the mean EQR scores among different water bodies, which explained 34.4% and 44.5% of total variability for POMI 14 and POMI 9 respectively (Table 1). Among the crossed intercepts variability was low among years (≈5% of total variability) and in particular among surveyors (<1% of total variability) for both POMI 14 and POMI 9 (Table 1). This corresponded to a minimum probability of misclassification of <2% for both factors under each version of POMI (Fig. 2c and d). 'Depth', in contrast generated relatively high uncertainty, with mean EQR values among depths of 5–17 m explaining 26.4% and 22.7% of the total variance

Table 1
Results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed POMI EQR scores analysed as a function of six random effects. POMI 14 (P14) and POMI 9 (P9) were analysed separately. Colon between factors represents nesting (i.e. Site:WB signifies that site is nested within water body).

Groups	Name	Variance		Std. dev.		P_{samp}	
		P14	P9	P14	P9	P14	P9
Zone:(Site:WB)	(Intercept)	0.002	0.005	0.039	0.070	3.5	11.6
Site:WB	(Intercept)	0.002	0.000	0.044	0.009	4.6	<1
WB	(Intercept)	0.015	0.019	0.121	0.137	34.4	44.5
Year	(Intercept)	0.002	0.002	0.046	0.045	4.9	4.8
Surveyor	(Intercept)	0.000	0.000	0.003	0.005	<1	<1
Depth (5–17 m)	(Intercept)	0.011	0.010	0.106	0.098	26.4	22.7
Residual		0.011	0.007	0.106	0.083	26.3	16.10
Depth[a] (10–17 m)	(Intercept)	0.000	0.00	0.000	0.00	<1	0

[a] Depth represents the variability in mean EQR scores explained by depths between 10 and 17 m and is based on the same analysis, without 5 m sites.

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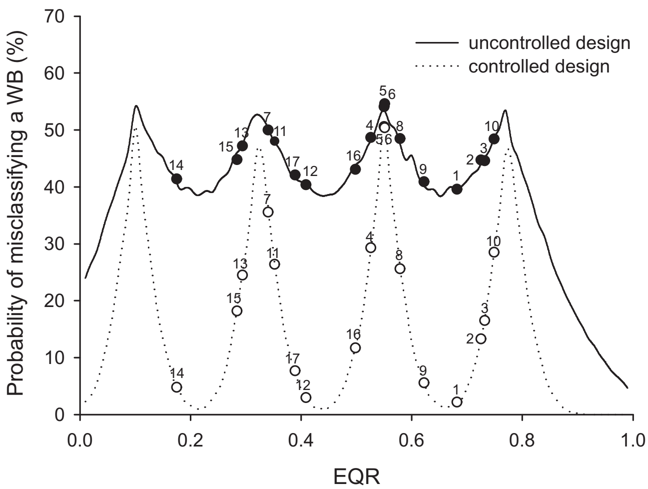

Fig. 3. Probability of misclassifying the ecological status class given the total variability among mean EQR values calculated by POMI 14 based on a 'controlled sampling design' and an 'uncontrolled design'. The controlled design represents among site variation and among depth (10–17 m) variation. The uncontrolled design represents cumulative variation, among zones, among sites, among years, among depths (5–17 m) and among surveyors. Full and open circles represent the actual probability of misclassification for 17 Catalonian coastal water bodies. Numbers represent the water body (Fig. 1). Probabilities are based on the 2008 data series.

for POMI 14 and POMI 9 respectively and a minimum probability of misclassification of >25% for both versions of POMI (Fig. 2e).

The total within-water body variation based on the annual monitoring program of Catalonian *P. oceanica* meadows was SD = 0.04 for the 'controlled design' (Eq. (5)) and SD = 0.13 for the 'uncontrolled design' (Eq. (6)). The probability of misclassifying the ecological status class of a water body ranged from a minimum of 1% for the controlled design, compared to a minimum of 38.4% probability of misclassification for the uncontrolled design. The stark difference in uncertainty between the two sampling designs is made evident by observing the superimposed probabilities of misclassification of the EQR scores from 2008 (Fig. 3). Water bodies with an EQR score close to the centre of the status class range (e.g. 9, 11 and 13) vary by over 35% in their probability of misclassification. Water bodies with an EQR close to the status class boundary (e.g. 5), however, are prone to a high probability of misclassification ($\approx$ 50%) irrespective of the sampling design.

4. Discussion

Identifying and gauging the sources of uncertainty inherent in the assessment of ecosystem health is critical in monitoring programs, especially when these programs can result in management decisions with high social and economic costs. To the best of the authors' knowledge, the current study is the first uncertainty analysis of its kind to have been conducted in coastal marine systems within the WFD member states. Overall, among the five factors examined, variations in depth between 5 and 15 m added the highest levels of uncertainty to the ecological status classification of *P. oceanica* meadows, accounting for over 20% of total variability among EQR scores and approximately 60% of total variability in estimation within water bodies. Depth, therefore, should remain fixed or be controlled in monitoring programs based on *P. oceanica* (see Montefalcone et al., 2007, 2009, 2010 for examples). The high levels of uncertainty associated with classifying the status of a *P. oceanica* meadows based on monitoring at variable depths is consistent with previous reports on the effect of depth on seagrass meadows. Seagrass meadows vary dramatically between depths as a result of light attenuation (Duarte, 1991), nutrient availability (Alcoverro et al., 2001) and high rates of herbivory in shallow (5 m) depths compared to the deep sites (i.e. 15 m, Martinez-Crego et al., 2010; Prado et al., 2007; Tomas et al., 2005) which in turn can affect the leaf biomass (Olesen et al., 2002), shoot density (West, 1990) and epiphytic community structure (Martinez-Crego et al., 2010). A meadow which has been sampled at multiple depths, therefore, results in highly variable EQR scores, independent of any anthropogenic induced changes to the environment, thereby inhibiting the ability to assess the true ecological status of the system and greatly increasing the probability of misclassification.

For the remaining factors, the uncertainty surrounding estimates in ecological status classification, based on the POMI sampling design was very low within water bodies. This signifies that as long as depth is fixed at approximately 15 m, POMI can be a precise indicator of ecological health status at the water body scale along the Catalonian coastline and that greater spatial replication will not dramatically influence the precision of water body status estimations. However, certain factors are relatively easy to account for in a sampling design by increasing replication, without increasing the time or financial expense of the monitoring program. For example, monitoring multiple zones within a single meadow (i.e. at 0 m, 25 m and at 50 m along a transect) helps to absorb part of the within-meadow heterogeneity, by providing a more robust estimate of the values of the different metrics than if just a single point is monitored. As this replication is achievable within a single scuba dive, increasing the precision of ecological status estimates can be achieved without incurring greater costs. Increasing the sampling effort in order to increase the precision of other factors, however, will substantially increase the time and expense of a monitoring program, without greatly reducing uncertainty and, therefore, should be considered in light of local or regional constraints. For example, inter-annual variability over a 3-year period is relatively low, contributing just 12% to total within water body variability. If yearly sampling is not possible, due, for instance, to financial constraints, it should be taken into account that sampling once every three years should not greatly reduce the precision of ecological status estimates, based on the two POMI indices examined in the current study. Furthermore, POMI 9 and POMI 14 demonstrated similar estimates of uncertainty for each of the factors observed in the study. POMI 9 could, therefore, be a cost effective alternative to POMI 14 pending further studies to test its viability detecting meaningful ecological change.

It is important to keep in mind, however, that irrespective of the factor or index in question, if the observed EQR score falls close to a status class boundary the probability of misclassification may still be as high as 50%. For example, variability in mean EQR scores between different surveyors was extremely low, explaining less than 1% of total variability. In spite of such low variability, however, 'surveyor' recorded up to 50% probability of misclassification at status class boundaries. The low variability between surveyors observed in the current study contrasts with a similar study on macrophyte communities in freshwater streams, which found moderate to high probability of misclassification of water bodies among surveyors (15–40% at the status class mid-point, Staniszewski et al., 2006). Unlike in Staniszewski et al. (2006), however, POMI is not based in any taxonomic identification; rather, the only directly attributable source of variation among surveyors came from estimates of meadow cover. Raw differences in meadow cover estimates were relatively low among surveyors and combined with the 8–13 additional metrics to form POMI 9 and POMI 14, respectively, between surveyors variability is minimised in final EQR estimations.

The current study has demonstrated how certain factors influence the precision or uncertainty when classifying the ecological status classes for *P. oceanica* meadows. Such analyses, however, cannot describe the accuracy of the indices predictions. A functional index should be a balance between one that will detect change at practical spatial and temporal scales for management and one that will reduce uncertainty in classification. An index, for example, that only monitors meadow cover, may generate very low uncertainty, however, respond too slowly to changes in water quality to provide constructive feedback to managers. Future studies must continue to weigh up the tradeoffs between detecting meaningful change and reliable classification ecosystem health.

5. Conclusions

The findings from the current study indicate that variations in depth between 5 and 15 m contribute relatively high levels of uncertainty to the ecological status classification *P. oceanica* meadows. These results emphasise the importance of controlling the sampling depth in *P. oceanica* meadows monitoring programs. Low variability among zones within sites and among sites within water bodies, suggests that both POMI 14 and POMI 9 are precise monitoring indices at the scale of water bodies along the Catalonian coastline.

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Highlights

> Uncertainty estimation is a central element in WFD-compliant classification methods

> Uncertainty associated to POMI index changes depending on the region of application

> Uncertainty associated to POMI depends on the quality status of water bodies

> Monitoring programmes design must respond to the specific conditions of water bodies

Uncertainty analysis along the ecological quality status of water bodies: the response of the *Posidonia oceanica* multivariate index (POMI) in three Mediterranean regions

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Abstract

Uncertainty analyses allow the identification and quantification of the factors that contribute to the potential misclassification of the ecological status of water bodies, helping to improve the sampling design used in monitoring. Here we used a *Posidonia oceanica* multivariate index (POMI) bio-monitoring dataset covering a total of 81 sites distributed throughout 28 water bodies from the coast of Catalonia, Balearic Islands and Croatia to determine the levels of uncertainty associated with each region and how they change according to the quality status of water bodies. Overall, variability among sites (meadows) within water bodies was the factor that generated the greatest risk of misclassification among the three regions, within which the Balearic Islands had the lowest uncertainty, followed by Croatia and Catalonia. When water bodies classified in good/high quality were separated from those in moderate/poor status classes, we found that the latter displayed higher levels of uncertainty than the former.

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1. Introduction

The requirement of the Water Framework Directive (WFD; EC, 2000) to classify all surface water bodies according to their ecological status through the use of specific biological quality elements (BQE) has prompted the development of new methods to monitor the status of European aquatic ecosystems. The concept of "ecological status", as defined by the WFD, is the expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters (Bennett et al., 2011; Kelly et al., 2009), and is determined by monitoring and assessing biological indicators that are subsequently integrated into an index with the aim to detect temporal and spatial changes in the ecological status of water bodies. As such, an index is subject to spatial and temporal heterogeneity, as well as to human error throughout the sampling and analytical stages. Therefore, any expression of ecological status will need to have an associated measure of uncertainty (Kelly et al., 2009). Uncertainty analyses are a useful tool to identify the factors that contribute to the risk of misclassification of the ecological status class of water bodies (Clarke and Hering, 2006; Staniszewski et al., 2006), which, if undetected, could result in considerable social, economic and ecological losses to the region, as management decisions are taken on the basis of these classifications. The first available studies on WFD-compliant classification methods have detected and quantified the main sources of uncertainty inherent in the assessment of ecosystem health within their respective systems (Bennett et al., 2011; Kelly et al., 2009; Staniszewski et al., 2006) and provided cost-effective insights that can help to improve bio-monitoring programs.

Within coastal waters, the biological quality elements identified by the WFD include phytoplankton, macroalgae, marine angiosperms and benthic invertebrate assemblages, selected for their proven sensitivity to anthropogenic disturbances and, therefore, their potential as indicators of the ecological status within the water body. Seagrass ecosystems, and in particular the Mediterranean *Posidonia oceanica* meadows, provide ideal BQEs to monitor ecological status in coastal waters due to their recognised value as biological indicators (López y Royo et al., 2010; Martínez-Crego et al., 2008; Pergent et al., 1995; Pergent-Martini et al., 2005) and their ubiquitous distribution throughout the Mediterranean Sea (Procaccini et al., 2003). A number of WFD-compliant classification systems based on *P. oceanica* have been developed (Buia et al., 2005; Gobert et al., 2009; López y Royo et al., 2010; Romero et al., 2007) or are under development (Casazza et al., 2006). Among them, POMI (*Posidonia oceanica* Multivariate Index; Romero et al., 2007) is used to monitor, evaluate and classify the ecological status of coastal water bodies along the Catalonian coast since 2005. More recently, it has been applied to other Mediterranean regions like the Balearic Islands and Croatia. A detailed analysis of a 7-year dataset, covering 30 sites along 500 km of the Catalonian coastline by Bennett et al. (2011) identified the main factors contributing to the uncertainty of the ecological status classification of water bodies when using POMI. Among the 5 considered sources of uncertainty (zones within a meadow, meadows within a water body, depth, year and surveyor), the study concluded that in order to maximize precision of POMI as an indicator of ecological health status at the water body scale, depth must be fixed and spatial heterogeneity (at among- and within-meadow scales) must be captured by means of an adequate replication.

Spatial replication is essential when monitoring seagrass meadows because a single water body may contain multiple, spatially independent meadows that typically display horizontal heterogeneity (e.g. zones of different density, structure, etc.). Even though different meadows within a water body should reflect similar ecological statuses, their natural heterogeneity over multiple spatial scales calls for a certain degree of replication smoothing among-meadow heterogeneity and providing a more robust estimate of the ecological status than that would be obtained if using a single meadow to monitor a water body. Adequate spatial replication has been found to be essential to minimize the risk of misclassification in other contexts, such as diatoms from lakes and rivers (Kelly et al., 2009). The power curves calculated in Kelly et al. (2009) suggest that the variability changes along the ecological quality gradient, which means that the uncertainty associated to the classification system would depend on the quality status of water bodies. This phenomenon may have drastic implications for monitoring and management plans and it requires further investigation.

Here we analyse the probability of misclassification of seagrass meadows based on a POMI sampling design in 3 locations largely representative of the Mediterranean eco-region, where *P. oceanica* was selected as the only representative angiosperm BQE (Med-GIG, 2007). We also evaluate the contribution of the water body quality status classification to the level of uncertainty, and its implications for designing monitoring programmes. To do so, we use EQR (Ecological Quality Ratio) values from POMI bio-monitoring programmes that covered the Catalan coast, the Balearic Islands and Croatia. Our aim is to address the applicability of the POMI classification index to Mediterranean coastal waters as a basis to monitoring and inform management plans.

2. Material and Methods

2.1. EQR dataset

The data analysed in this study was obtained from the POMI bio-monitoring programmes that determined the ecological quality status of coastal water bodies (WB) between 2006 and 2008. We selected those WBs with a minimum of 2 replicated *P. oceanica* meadows, including a total of 7 WB in Catalonia (20 meadows), 18 WB in the Balearic Is. (44 meadows) and 3 WB in Croatia (17 meadows) (Fig. 1). The ecological status of each *P. oceanica* meadow was determined following the methodology described in Romero et al. (2007). In each meadow, 3 zones of similar depths were sub-sampled and metric values were averaged among the zones to address within-site variability (Bennett et al., 2011; Martínez-Crego et al., 2008; Romero et al., 2007). Within each zone, up to 14 biological metrics were determined for POMI: 5 representative of the physiological level (phosphorous, nitrogen and sucrose content and $\delta^{15}N$ and $\delta^{34}S$ isotopic ratios in rhizomes); 2 of the individual level (percentage of leaves with necrosis and shoot leaf surface area); 3 of the population level (meadow cover, shoot density and percentage of plagiotropic rhizomes); 1 of the community level (nitrogen content in the epiphytes) and 3 are pollution indicators (copper, lead and zinc concentration in rhizomes). Whereas the POMI of Catalonia and Croatia included all 14 metrics, only 5 were used in the Balearic Islands index (meadow cover, phosphorous and nitrogen content and $\delta^{15}N$ and $\delta^{34}S$ isotopic ratios in rhizomes). The metrics were integrated onto a single scale using Principal Component Analysis (PCA), and the resultant score of each meadow, on the first axis of the PCA, was then used to calculate its EQR' using the equation:

$$EQR'_x = (CI_x - CI_{worst}) / (CI_{optimal} - CI_{worst}) \quad (1)$$

where EQR' is the ecological quality ratio of site *x,* and CI_x, CI_{worst} and $CI_{optimal}$ are the scores of site *x*, of the worst reference site and of the optimal reference site on the first component respectively. In the current study, the optimal and worst reference conditions are calculated as the mean value of the best and worst three scores for each metric respectively for each region, except for the worst reference condition in the Balearic Islands where it was calculated from the worst three scores recorded in the Balearic Islands and Catalonia. Note that optimal conditions reflected either high or low scores depending on the metric in question (e.g. high shoot density is optimal whilst low lead concentration is optimal; Romero et al., 2007).

The ecological status of each site is then classified into one of five classes from 'high' to 'bad', set within the 0 – 1 EQR scale, following Romero et al. (2007). Because of its sensitivity to anthropogenic disturbance, *P. oceanica* does not survive in extremely bad conditions, therefore, no matter how degraded the meadow, all sites used in the current study were considered better than "bad", implicitly setting "bad" as the absence of *P. oceanica* in sites where it should otherwise be present. The upper four status class boundaries were determined by dividing the remaining EQR scale (0.1 – 1) into four equal categories. The EQR of each site was, therefore, classified into one of the upper four status classes using the equation:

$$EQR_x = (EQR_x' + 0.11) / (1 + 0.10) \qquad (2)$$

2.2. Variance components

The current study investigated how the variability among meadows within a water body is influenced by: 1) the sampling region (Catalonia, Balearic Islands and Croatia) and 2) the quality status of water bodies (between good/high and moderate/poor). Since the lowest level of spatial replication in the bio-monitoring dataset was at the water body scale, variance among meadows within water bodies was quantified by the residual term of a linear mixed effects model in the nlme package of R (Pinheiro and Bates, 2000; Pinheiro et al., 2005; Version 2.12.2, R_Development_Core_Team 2009). Initially, the model was applied to the POMI bio-monitoring data from each region independently (question 1). After that, it was applied first to all data pooled from the three regions and then to data from water bodies classified in “good/high” quality status separately from those in “moderate/poor”, in an attempt to determine the influence of water body quality status in the variability among meadows (question 2). Pooling data from the three regions together delivered a sufficient number of water bodies in each of the two ecological statuses considered to ensure a robust variance extraction, which could not otherwise have been possible in each region independently. In all cases, water body was considered as a random effect. Thus, total variability (σ^2_T) was explained by the variance due to differences in mean EQR values among water bodies (σ^2_{WB}) and by the variation among sites within water bodies included in the residual variance of the model ($\sigma^2_{residual}$):

$$\sigma^2_{total} = \sigma^2_{WB} + \sigma^2_{residual} \qquad (3)$$

The proportion of total variance (P_{samp}) explained by each factor was given by the equation, following Clarke et al. (2006):

$$P_{samp} = 100\ \sigma^2_{x} / \sigma^2_{T} \qquad (4)$$

It is important to clarify that variation among water bodies (σ^2_{WB}), whilst important in the analysis of variance components, is not presented in the results of this study because by definition they should differ in their ecological status. All data satisfied the assumption of homogeneity of variance based on plots of the residuals against the fitted EQR values; therefore, transformation of data was unnecessary.

2.3. Analysis of uncertainty

Once the variation in mean EQR scores among meadows within a water body for all data combinations was calculated (see above), the uncertainty in ecological status classification was estimated using WISERBUGS (WISER Bioassessment Uncertainty Guidance Software®; Clarke, 2010). WISERBUGS helps determine whether an observed ecological status classification is indeed the most probable classification for a particular site, given the inherent sources of variability. WISERBUGS sums the observed value for a given site with a random standard normal deviate, of the known SD, with a mean of zero (Clarke and Hering, 2006). It repeats this simulation 10^4 times to produce a frequency distribution of possible EQR values for the particular site or water body. The simulated EQR values are grouped into their corresponding status classes, from which the probability of misclassifying the original observed value can be determined. Because the current study was interested in the uncertainty in classification generated by a particular factor (rather than the probability of misclassifying individual sites), the simulation was repeated for the full range of possible observed EQR values (0 - 1).

3. Results

3.1. Uncertainty associated with inter-meadow variability in POMI classification across different geographical regions

When the variance among meadows within water bodies was extracted independently from the three regions under study, remarkable differences were found. The Balearic Islands region displayed the lowest variability within water bodies (SD = 0.081; Table 1), followed by Croatia (SD = 0.118; Table 1) and Catalonia (SD = 0.128; Table 1) resulting in large differences in the uncertainty associated with each region. In all cases, the probability of misclassification peaks when a site's observed EQR score is very close to the boundary between two status classes, reaching values of 50% or more, and is lowest when the observed EQR falls in the middle of a status class. The magnitude of the highest and lowest uncertainty values differed greatly among regions as a result of the differences in the variance calculated. The Balearic Islands was the region with the lowest probability of misclassification (15 to 50%, Fig. 2). Croatia and Catalonia showed higher probability of misclassification, with levels ranging from 35 to >50 % and from 40 to >50 % respectively (Fig. 2).

3.2. Uncertainty associated to inter-meadow variability in POMI classification depending on water body quality status

In the first case, variance among meadows within water bodies was high when it was extracted from the whole dataset of the three regions (SD = 0.108; Table 2). However, drastic differences were found when the variance among meadows was extracted separately according to the quality status of water bodies ("good/high" and "moderate/poor"). Water bodies classified in poor and moderate quality statuses displayed higher variability than those in good and high (SD = 0.129 and 0.098 respectively; Table 3). This resulted in drastic differences in the levels of uncertainty along the quality gradient (Fig. 3). Following the pattern of the extracted variance, higher uncertainty levels were found in those water bodies classified as moderate or poor status, displaying maximum levels of ca. 55% in the boundary between classes and minimum of 45% in the middle of a status class (Fig. 3). On the other hand, those water bodies classified in either good or high quality status showed lower levels of uncertainty, ranging from maximum values of 50% to minimum of 20% (Fig. 3).

4. Discussion

Uncertainty analyses allow the identification and quantification of the factors that affect the risk of misclassification when assessing ecosystem health through the use of biological indicators. Applied to monitoring programs, this knowledge can be used to guide management decisions that would help to maximize the confidence of estimations, like the number and distribution of replicates that should be taken. In the current study, we observed that across different regions of the Mediterranean, variability among meadows added different levels of uncertainty to the ecological status classification of water bodies when using the *Posidonia oceanica* multivariate index (POMI). All regions displayed a similar uncertainty pattern across the quality gradient, with maximum levels in the boundary between two status classes (50% or more) and minimum when the EQR score falls in the middle of a status class, as previously reported by Bennett et al. (2011). The Balearic Islands displayed the lowest levels of uncertainty, followed by Croatia and lastly, Catalonia, where the highest uncertainty levels were found. Besides, when the within-water body uncertainty was analysed separately according to the quality status of water bodies, our results show that those classified in good/high status displayed lower uncertainty levels than those classified as moderate/poor status. Since this study focused on the variance within water bodies, an increase in the uncertainty associated must be related to a higher degree of heterogeneity at this scale, which means a higher variability among meadows within a water body.

In the Water Framework Directive, the delimitation of the spatial extent of coastal water bodies is done according to the water quality and anthropogenic pressures to which the coastline is exposed, independent of the number, or status, of seagrass meadows within the area (EC, 2000). This definition poses a challenge since it should be based on sound scientific underpinnings, and accommodates the management requirement that pollution abatement measures are effective and verifiable at the water body scale (Ferreira et al., 2006). In the case of POMI, the ecological status of a water body derives from the averaged EQR scores of the different *P. oceanica* meadows monitored within it. Our results indicate that care should be taken when dealing with water bodies subjected to human pressures, since their effects (from discrete point sources or from diffuse non-point contamination; Badalamenti et al., 2006; Boudouresque et al., 2009; Orth et al., 2006) may not be uniformly distributed among the different *P. oceanica* meadows included in a water body, widening the natural range of variability at this scale and potentially raising the uncertainty associated to the quality status classification. This finding has important implications for the design of monitoring programmes based on *P. oceanica*. On the one hand, it indicates that a greater replication effort should be assigned to those water bodies classified in moderate/poor/bad status, in order to capture the extra spatial variability coming from the effects of human pressures. On the other hand, it may be also a first warning that the spatial extent of water bodies may need to be redefined in some extreme cases. In effect, when differences in mean EQR values among different meadows of the same water body are excessively high, an adequate spatial replication design will not be able to reduce the uncertainty associated to the classification system. A redefinition of the spatial extent and number of water bodies is strongly recommended in such cases.

The differences in the uncertainty levels of the POMI classification index found among the three regions considered in this study may be largely related with the differences in human pressures among them. Thus, the lower uncertainty levels found in the Balearic Islands can be related to the generally low human affectation of water bodies, as revealed by the fact that almost all meadows present high and homogeneous EQR values (a total of only 2 sites out of 44 in moderate status, resulting in only 1 WB out of 18 in moderate status). The low levels of uncertainty found in this region indicate that both the spatial replication design and the water body spatial extent are well defined.

On the other hand, *P. oceanica* meadows from Catalonian and Croatian water bodies show a wider range of impacts by human pressures (Catalonia: a total of 14 sites out of 20 below the good status, resulting in 3 WB out of 7 in moderate/poor status; Croatia: a total of 5 sites out of 17 below the good status, resulting in 1 WB out of 3 in moderate status), which widens the natural range of variability among meadows and promotes higher levels of uncertainty in the classification system with the actual spatial replication design and water body spatial extent definition. In water bodies where this heterogeneity in EQR values among meadows is low, a greater replication effort should be assigned to account for the extra spatial variability and reduce the risk of misclassification. In extremely heterogeneous water bodies, which include meadows with highly contrasting EQR scores (0.400 to 0.847 in water body CAT14 of Catalonia; 0.237 to 0.685 in water body O423-BSK of Croatia), a redefinition of the spatial extent is necessary to ensure that the classification of the spatial extent of coastal water bodies reflects appropriately their water quality and the anthropogenic pressures to which the coastline is exposed, as stated in the Water Framework Directive (EC, 2000).

The assessment of the variation of uncertainty levels according to the water body quality gradient and the consequences for the classification of ecological status must be examined further to robustly use biological communities to assess ecological status of aquatic ecosystems.

5. Conclusions

When designing monitoring programs to classify the ecological quality status of water bodies using the *Posidonia oceanica* Multivariate Index (POMI), sampling effort must be set according to the specific conditions of each water body, region and associated human pressures. A greater replication effort must be assigned to regions and water bodies that are subject to significant human pressures (high population density, industrialization, etc.), spanning a sufficient number of meadows within each water body to provide a robust and reliable average value of their quality status. In water bodies where only a single or few meadows exist, uncertainty estimates should be calibrated with water bodies of similar ecological status. Water bodies encompassing meadows with highly contrasting EQR scores need be split into different water bodies to clearly delineate areas affected by human impacts from those less influenced.

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Figure 1: Map of the location of the sampling sites (meadows) and water bodies along the Catalonian, Balearic and Croatian coasts, from where bio-monitoring POMI data was obtained.

Figure 2: Probability of misclassifying the ecological status class associated to the variability among sites within water bodies (included in the residual variance of the model) for the three regions under study (Catalonia, Balearic Is. and Croatia). Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.099; Poor = 0.1 – 0.324; Moderate = 0.325 – 0.54; Good = 0.55-0.774 and High = 0.775 – 1.

Figure 3: Probability of misclassifying the ecological status class associated to the variability among sites within water bodies (included in the residual variance of the model) for the data pooled from the three regions under study and according to their quality status. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.099; Poor = 0.1 – 0.324; Moderate = 0.325 – 0.54; Good = 0.55-0.774 and High = 0.775 – 1.

Table 1: Results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed POMI EQR scores analysed as a function of water body for each region independently.

Region	Groups	Name	Variance	Std. Dev.	Total variance (%)
Catalonia	Water body	(Intercept)	0.012785	0.113070	43.8
	Residual variance within water bodies		0.016374	0.127962	56.2
Balearic Is.	Water body	(Intercept)	0.006080	0.077971	47.8
	Residual variance within water bodies		0.006627	0.081407	52.2
Croatia	Water body	(Intercept)	0.006023	0.077605	30.2
	Residual variance within water bodies		0.013899	0.117891	69.8

Table 2: Results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed POMI EQR scores from Catalonia, Balearic Is. and Croatia pooled data analysed as a function of water body.

Groups	Name	Variance	Std. Dev.	Total variance (%)
Water body	(Intercept)	0.019051	0.138027	62.1
Residual variance within water bodies		0.011626	0.107822	37.9

Table 3: Results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed POMI EQR scores from Catalonia, Balearic Is. and Croatia pooled data analysed as a function of water body separately according to their quality status.

Quality	Groups	Name	Variance	Std. Dev.	Total variance (%)
Good/High	Water body	(Intercept)	0.004944	0.070314	34.0
	Residual variance within water bodies		0.009582	0.097888	66.0
Moderate/Poor	Water body	(Intercept)	0.004386	0.066228	20.8
	Residual variance within water bodies		0.016728	0.129336	79.2

Figure1
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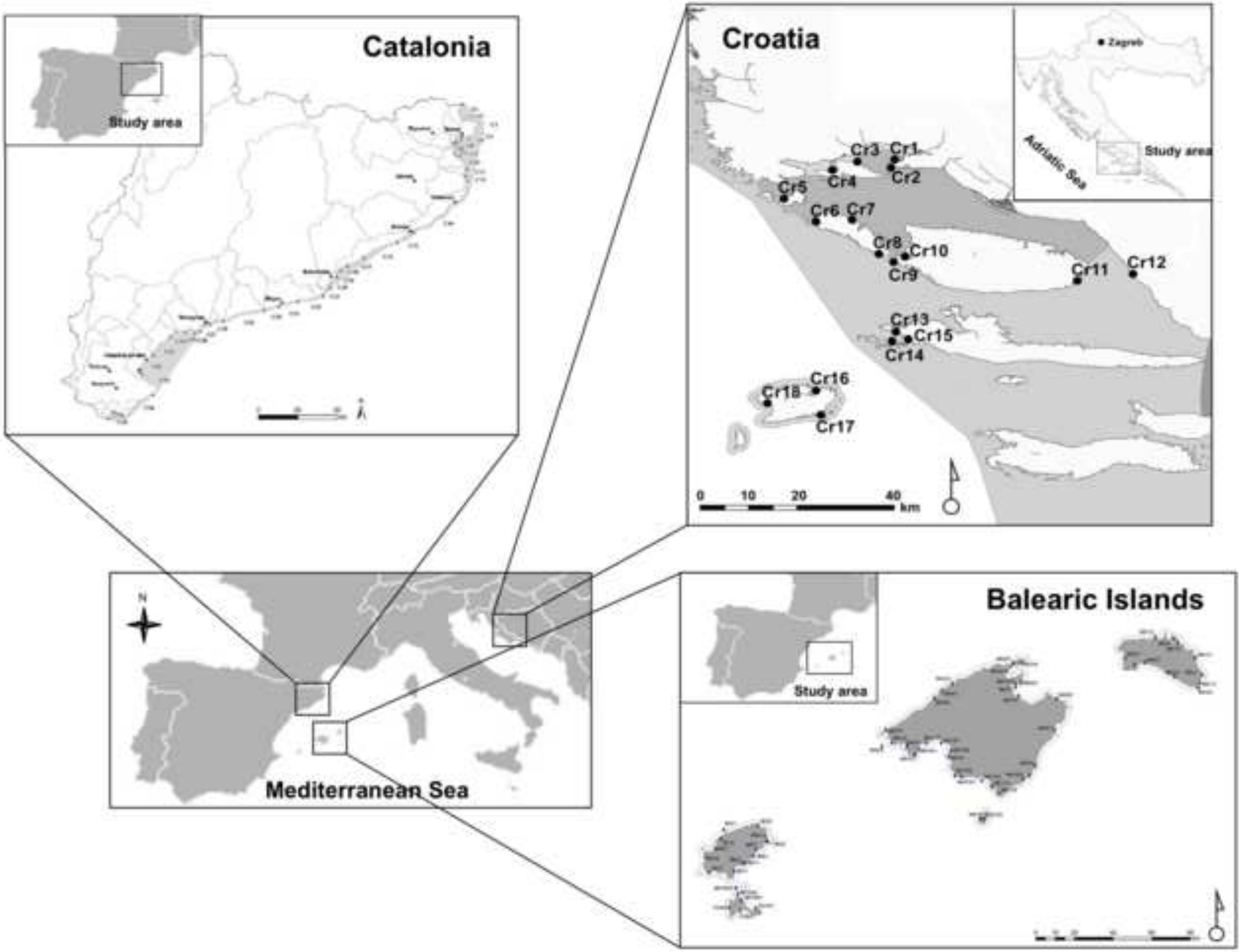

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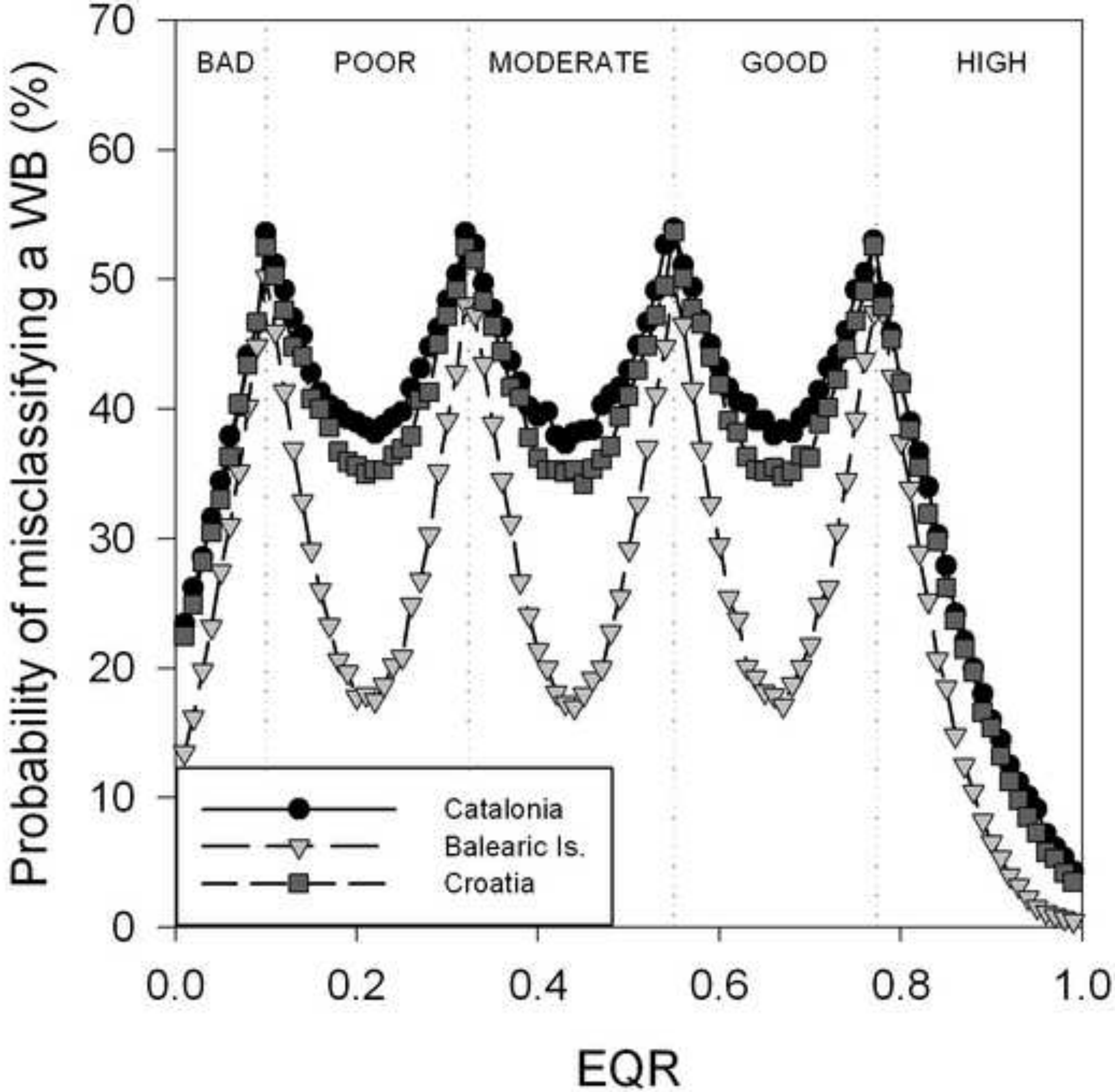

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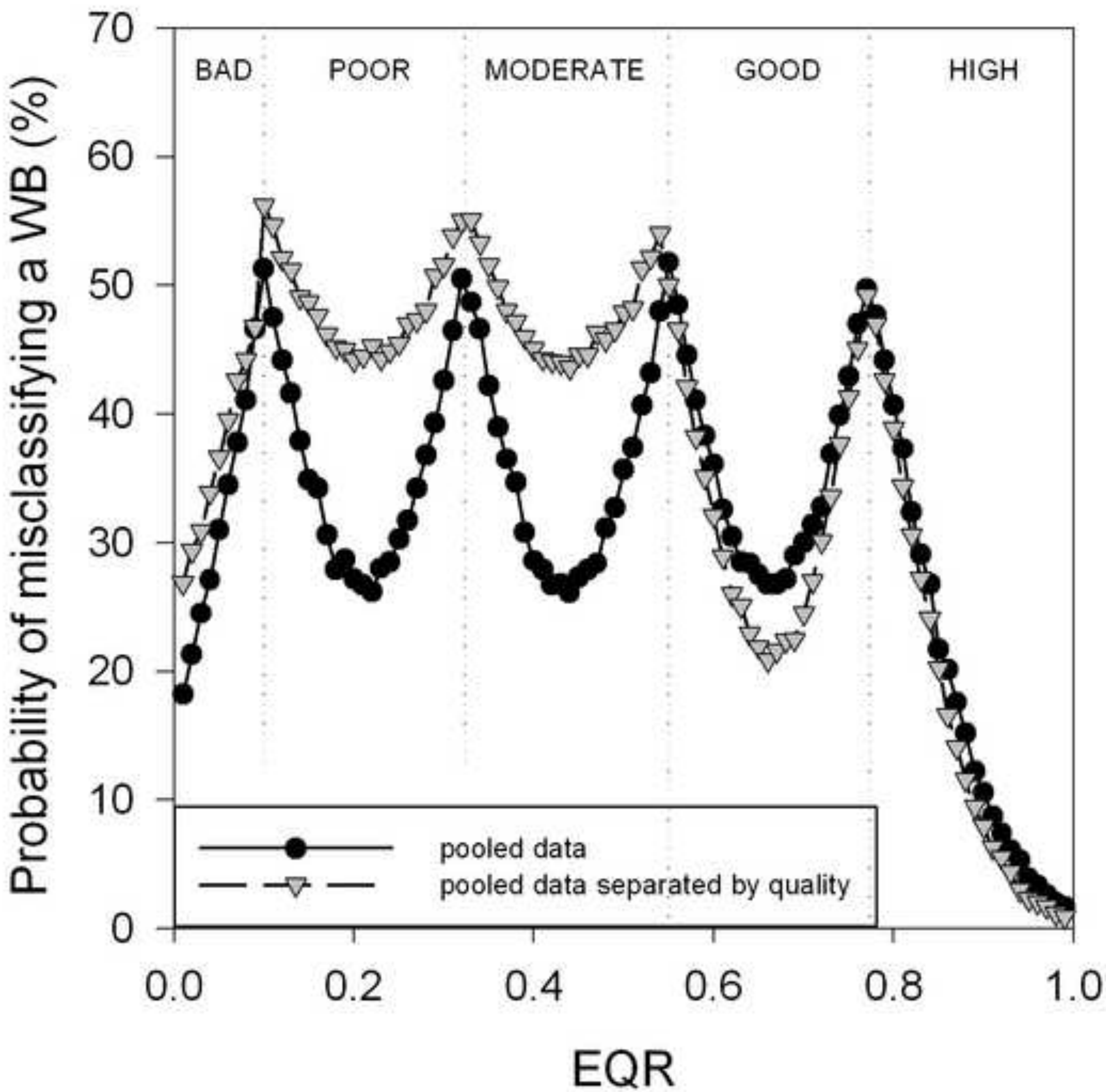

Exploring the robustness of different macrophyte-based classification methods to assess the ecological status of coastal and transitional ecosystems under the WFD

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Abstract

Identifying and quantifying the factors that contribute to the potential misclassification of the ecological status of water bodies is a major challenge of the next phase of WFD implementation, and one of the main goals of the WISER Project. The present study compiles extensive bio-monitoring data from several macrophyte-based classification methods developed by different EU Members, which include data addressing spatial, temporal and human-induced sources of variability. Through the application of uncertainty analysis, we determined that factors related to the spatial scale of sampling added the highest levels of uncertainty to the ecological status classification of water bodies, probably due to the high horizontal and vertical heterogeneity displayed by macrophyte communities. On the contrary, the uncertainty associated to temporal variability and human-induced errors was very low, for which the frequency of sampling could be decreased and the number of surveyors minimized without greatly reducing the precision of ecological status estimates.

Keywords: biological quality elements; Water Framework Directive; uncertainty;

1. Introduction

The requirement of the EU Water Framework Directive (WFD; Directive 2000) to classify all surface water bodies according to their "ecological status" has precipitated a fundamental change in management objectives from merely pollution control to ensuring ecosystem integrity as a whole (Hering et al. 2010). The concept of "ecological status", as defined by the WFD, is the quality of the structure and functioning of aquatic ecosystems associated with surface waters (Bennett et al. 2011). Rather than focus only on limited aspects of chemical quality, the WFD establishes that the ecological status has to be determined by monitoring and assessing the so-called Biological Quality Elements (BQEs; Moss 2007, Lopez y Royo et al. 2011), which must be integrated into an index with the aim to detect temporal and spatial changes in the quality of water bodies (Bennett et al. 2011).

However, this innovativeness comes with a number of substantial challenges for ecologists in requiring complex and dynamic biological communities to be quantified into a single numeric score, for reference conditions to be established from which to measure the degree of change, and for this all to be carried out within a large number of water body types (Hering et al. 2010). The development of methods for water body quality assessment fulfilling the complex requirements of the WFD has been faced by each Member State individually (Søndergaard et al. 2005), resulting in the appearance of a wide variety of methods throughout Europe that differ greatly in the way of defining reference conditions, type vs. site-specific assessment, the number and nature of indices (metrics) used, etc. (Hering et al. 2010).

The WISER Project (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery; www.wiser.eu) was conceived to evaluate the robustness and reliability of the different indices developed by the EU members, addressing all water categories, organism groups and environmental stressor types. This is to be done mainly through the use of uncertainty analysis, a powerful tool that allows the identification of the factors contributing to the potential misclassification of the ecological status class of water bodies (Clarke and Hering 2006, Staniszewski et al. 2006). The estimation of uncertainty is a central element in WFD-compliant assessment methods, since they are based on biological communities that show both spatial and temporal heterogeneity, and because errors will be introduced during sampling and analytical stages (Clarke and Hering 2006, Carstensen 2007, Kelly et al. 2009). If the major sources of variability are known, they can potentially be minimised through the re-design of sampling schemes (additional sampling sites or frequency), through improved training by operating procedures, CEN (European Committee for Standardization) guidance, taxonomic training or through the use of model-based assessment methods (Pont et al. 2009). For this reason, ecological status classification results should always be given in terms of probabilities depending upon the variability associated with these communities over time and space (Hering et al. 2010). However, only a small proportion of classification methods have put this into practice and the uncertainty analyses available in the literature are relatively scarce at the moment (but see Staniszewski et al. 2006, Kelly et al. 2009, Bennett et al. 2011).

The objective of this contribution is to determine which sources of variability (factors) associated with the sampling design of a subset of different monitoring programmes (6 different classification methods) based on macrophytes (either macroalgae or seagrasses) developed by EU Member States under the Water Framework Directive (Norway, Denmark, Bulgaria, Spain, Croatia, Italia and Portugal), most greatly influence ecological status classifications of these biological communities. The analyses will be based on EQR datasets of either official or non-official bio-monitoring programmes of the different indices from which a data set including enough temporal and spatial replication was available, and the factors analysed will include spatial scales of sampling (variability among zones within a site, among sites within a water body, variability among regions and variability among depths), the temporal scale of sampling (variability among years) and the human-associated source of error (variability between surveyors). These factors represent some the key sources of variability associated with the design and implementation of a bio-monitoring program, and highlight how certain elements of a sampling design can influence the reliability and robustness of the ecological status classification of coastal water bodies. With this approach, we try to gain insight into the current status of these methodologies proposed for European waters under the WFD and detect their main weaknesses to provide robust foundation for monitoring as well as guide decision in management plans.

2. Material and Methods

2.1. Indices included in the study

In this study, we analysed data from bio-monitoring programs of 8 different indices based on macrophyte metrics and developed under the WFD, that are currently applied to monitor the ecological quality of coastal water bodies in different regions of Europe (Fig. 1). The indices included in this study and their corresponding regions of application and water types are: i) "Multi Species Maximum Depth Index" (North Sea - Norway, Coastal Waters), ii) "Eelgrass Depth Limit" (Baltic Sea - Denmark, Costal Waters), iii) "*Posidonia oceanica* Multivariate Index" (Western Mediterranean - Spain and Croatia, Coastal Waters), iv) *Cymodocea nodosa* Multi-bioindicator Index (Western Mediterranean - Spain, Transitional Waters), v) Rocky Intertidal Community Quality Index (North Atlantic Ocean - Spain, Coastal Waters), vi) "Ecological Evaluation Index" (Adriatic Sea - Italy, Transitional Waters), vii) "Ecological Index" (Black Sea - Bulgaria, Coastal Waters) and viii) "Seagrass Quality Index" (Atlantic Ocean - Portugal, Transitional Waters). The indices differed in their target macrophyte species, from a list of specific macroalgae (MSMDI) to a single seagrass species (POMI), as well as in the nature and number of metrics used. Thus, whereas some indices included one single metric (e.g. lower depth limit, EDL), others were calculated integrating a series of attributes spanning different levels of organization (e.g. physiological, morphological, population and community levels, POMI). In the multimetric indices, there are also differences in the method used to integrate the variables, from a sum of metrics (EEI-c, EI, SQI) to ordination techniques to integrate the group of variables (Principal Component Analysis, POMI). Finally, one of the most important differences among indices is how the EQR range is split into the five quality status classes established by the WFD (bad/poor/moderate/good/high; Birk and Hering 2006). Whereas the EQR range is split into 5 equal classes in most of the indices (0.2/0.4/0.6/0.8 boundary class values for MSMDI, EI, SQI and CYMOX), some others present status classes of unequal wide due to particular methodological restrictions (EDL, POMI, RICQI and EEI). This fact may promote drastic changes in the uncertainty levels along the EQR range depending on the status class, and needs to be taken into account when analysing the risk of misclassification. All relevant information regarding the 8 indices included in the present study is summarized in Table 1.

2.2. Variance extraction

In the current study, the factors examined that potentially contribute to the uncertainty of the EQR estimations of coastal water bodies differ greatly among the 8 indices, especially due to differences in both the metrics used and their corresponding spatial and temporal sampling designs (Table 2). The total variance and variance components associated to each factor were estimated for all indices using a linear mixed effects model in the lme4 package of R (Bates 2005 and 2007, Version 2.10.1, R_Development_Core_Team 2009). When sufficient data was available, factors were treated as random intercepts, either nested or crossed depending on the index (Table 2). Note that the variability among water bodies, whilst important in the analysis of variance components, is not discussed in this study because by definition they should differ in their ecological status. Variance components were determined by calculating the proportion of the total variance (σ^2_T) explained by each individual factor. Thus, total variance in mean EQR values for each index was given by the sum of variances associated to each of the factors included in the model (σ^2_X) plus the variance not explained by the model (σ^2_R; Table

2). The proportion of total variance (P_{samp}; Table 2) explained by each factor was given by the equation, following Clarke et al. (2006):

$$P_{samp} = 100\ \sigma^2_X / \sigma^2_T \qquad (1)$$

Posteriorly for each index, the extracted variances were grouped into four main sources of uncertainty: i) temporal scale of sampling (variability among years), ii) spatial scale of sampling (including variability among zones within a site, among sites within a water body, variability among regions, variability among depths, etc.), iii) human-associated source of error (variability among surveyors) and iv) the residual term of the analysis (the variance in mean EQR values not explained by the model) in order to allow a further comparison of the results among indices that would help drawing general conclusions about these macrophyte-based classification methods (see Table 2).

All data satisfied the assumption of homogeneity of variance based on plots of the residuals against the fitted EQR values; therefore, no transformation of the data took place.

2.3. Uncertainty analysis

Having calculated the variation in mean EQR scores for all factors within each index, the uncertainty in ecological status classification was estimated using WISERBUGS (WISER Bioassessment Uncertainty Guidance Software®, Clarke 2010). WISERBUGS helps determine whether an observed ecological status classification is indeed the most probable classification for a particular site, given the inherent sources of variability. WISERBUGS sums the observed value for a given site with a random standard normal deviate, of the known SD, with a mean of zero (Clarke and Hering 2006). It repeats this simulation 10^4 times to produce a frequency distribution of possible EQR values for the particular site or water body. The simulated EQR values are grouped into their corresponding status classes, from which the probability of misclassifying the original observed value can be determined. Because the current study was interested in the uncertainty in classification generated by a particular factor (rather than the probability of misclassifying individual sites), the simulation was repeated for the full range of possible observed EQR values (0 - 1).

3. Results

3.1. Analysis of the uncertainty associated to the ecological status classification

Depending on the index, the factors examined displayed different levels of uncertainty in the ecological status classification of water bodies. Generally for all factors, the probability of misclassification peaks when a site's observed EQR score is very close to the boundary between two status classes, usually around 50%. In contrast, when the observed EQR falls in the middle of a status class the probability of misclassification declines to the minimum. Probabilities of misclassification >50% may indicate that the associated variability is actually higher than the EQR range of the status class. The magnitude of these maximum and minimum uncertainty levels differ greatly among factors and indices as a result of the differences in the variance extracted. In summary, the higher the variability, the higher its probability of misclassification even in the centre of the status class ranges.

i. Multi Species Maximum Depth Index (MSMDI)

In this index, all the examined factors showed a low variability in the mean EQR scores, which determined also low associated probabilities of misclassification. On the one hand, the factors “year”, “region” and “surveyor” displayed almost negligible levels of variability, explaining only 2.2%, 0.0% and 4.0% of total variance respectively (Table 3). This corresponded to a minimum probability of misclassification of 0% and maximum of 50% for each of these factors (Fig. 2). Even still low, variability in the mean EQR scores among different sites was higher, explaining up to 24.4% of total variance (Table 3) and resulting in levels of uncertainty ranging from 6% to 50% (Fig. 2). Finally, the variability not explained by the model represented up to 27.6% of total variance, for which the levels of uncertainty associated to unknown sources ranged from 7% to 50% (Table 3, Fig. 2).

ii. Eelgrass Depth Limit (EDL)

All factors analysed for this index showed relatively high variability, determining also high probabilities of misclassification. In this case, however, the levels of uncertainty associated to each factor increase along the EQR range as the width of the status classes narrows (0.25/0.5/0.74/0.9 boundary values; Fig. 3). The factor “year” displayed the lowest levels of variability in the mean EQR scores, representing 9.7% of total variability (Table 4). Its corresponding probabilities of misclassification included minimum values from 16% to 36% and maximum of 50% to 54%, following the EQR range (from 0 to 1; Fig. 3). The factors “region” and “site” showed a higher and similar variability in the mean EQR scores observed, explaining 30.2% and 24.4% of total variance respectively (Table 4). As a result, the probability of misclassification in the centre of a status class ranged from 40% to 58% along the

EQR range (from 0 to 1), whilst in the boundary between two status classes ranged from 54% to 64% (approximate values for the two factors; Fig. 3). For the residual term of the analysis, it represented up to 30.4% of total variance, for which high levels of uncertainty were associated to unknown factors for this index (minimum levels from 42% to 60% and maximum from 60% to 65% along the EQR range; Fig. 3).

iii. Posidonia oceanica Multivariate Index (POMI)

In this index, great differences in the variance and the associated risk of misclassification were observed among the several analysed factors. On the one hand, the factors “year”, “site”, “zone” and “surveyor” displayed very low variability, representing only 4.9%, 4.5%, 3.0% and 0% of total variance each (Table 5). As a result, their associated probability of misclassification was also low, ranging from minimum levels of 2.6%, 1.9% and 0.4% for “year”, “site” and “zone” respectively, to maximum levels of c.a. ≤50%; since the variance of the factor “surveyor” was negligible (σ^2<0.000000), the uncertainty associated to this factor was considered 0% along the whole EQR range (Fig. 4). On the other hand, the highest variability was observed in the mean EQR scores among regions and depths, which explained 29.8% and 25.8% of total variance respectively (Table 5). This corresponded with an also high probability of misclassification associated to these factors, from minimum values of 36% and 33% to maximum of 54% and 53% for “region” and “depth” respectively (Fig. 4). The residual term of the analysis represented up to 17.1% of total variance, determining relatively high levels of uncertainty due to unknown factors (from 24% to ≤50%; Fig. 4).

iv. Cymodocea nodosa Multi-bioindicator Index (CYMOX)

All factors included in this index showed a low variability in the mean EQR scores, which determined also low associated probabilities of misclassification. The factors “year” and “region” displayed negligible levels of variability (σ^2<0.000000; Table 6), for which the risk of misclassification associated to these factors was considered 0% along the whole EQR range (Fig. 5). The variance associated to the factor "site" was also low, representing only 2.2% of total variance and determining low levels of uncertainty ranging from 0% in the middle of a status class to 50% in the EQR values that separate status classes (Fig. 5). Only the variability associated to the residual term of the analysis was high, representing up to 21.3% of total variance and corresponding with also high uncertainty levels along the EQR range (from 45% to 55%; Fig. 5).

v. Rocky Intertidal Community Quality Index (RICQI)

In this index, the lack of replication among different water bodies may determine the high variability associated to the factors included in the biomonitoring program. On the one hand, variability among years was relatively high, representing 14.4% of total variance (Table 7) and determining levels of uncertainty that ranged from 17% to 50% (Fig. 6). On the other hand, variance associated to the spatial factor "site" was extremely high, representing 73% of total variance (Table 7) and displaying uncertainty levels between 54% and 61% along the whole EQR range (Fig. 6). Finally, the residual term of the analysis accounted for 12.6% of total variance (Table 7), and with uncertainty levels that ranged from 14% to 50% (Fig. 6).

vi. Ecological Evaluation Index (EEI-c)

In this index, variability among sites was negligible (σ^2<0.000000; Table 8), for which the risk of misclassification associated to this factor was 0% along the whole EQR range (Fig. 7). In contrast, the residual variance in mean EQR values was high, accounting for 30.5% of total variance (Table 8) and determining high levels of uncertainty that remained ≥50% almost along the whole EQR range (Fig. 7). The increasing width of the status classes along the EQR range (from 0 to 1) promoted that the general risk of misclassification decreased from "poor" to "high" status.

vi. Ecological Index (EI)

In this index, the factor “year” represented only 1% of the total variance (Table 9), which corresponded to a minimum risk of misclassification of 4.3% in the boundary between two status classes and a maximum of 51% when the EQR score falls in the middle of a status class (Fig. 8). In contrast, high levels of variability were observed in the mean EQR scores among sites and among depths, explaining 25% and 37% of total variance respectively (Table 9). Their corresponding probability of misclassification was extremely high, with levels ranging from 64.2% to 68.1% for “site” and from 69.4% to 72.2% for “depth” (Fig. 8). Finally, the residual variance was low, representing only 3% of the total variance and accounting for a risk of misclassification that ranged from 16.2% to 50.7% (Fig. 8).

vii. Seagrass Quality Index (SQI)

All the factors analysed for this index displayed a really low variability. On the one hand, variability among samples was negligible (σ^2<0.000000; Table 10) and its corresponding risk of misclassification remained 0% all along the EQR range (Fig. 9). On the other hand, variability in the mean EQR scores among years and zones was also low, representing 3% and 5.8% of total variance (Table 10) and with a probability of misclassification associated that ranged from 0% to 50% (Fig. 9). Even the residual term of the analysis, which accounted for 91.2% of total variance (Table 10), presented a low variability that promoted also a low risk of misclassification of 0% in the centre of a status class up to 50% at the boundary between classes (Fig. 9).

3.2. Main common sources of uncertainty among indices

For each index, the variances extracted for the different factors were grouped into four main sources of uncertainty: i) the temporal scale of sampling (variability among years), ii) the spatial scale of sampling (including variability among zones within a site, among sites within a water body, variability among regions, variability among depths, etc.), iii) human-associated sources of error (variability among surveyors) and iv) the residual term of the analysis (the variance in mean EQR values not explained by the model).

The spatial scale of sampling (excluding variability among water bodies) represented the main source of uncertainty, accounting for an average proportion of 35.9±10.7% of total variance among the different indices (mean±SE; see Table 11, Fig. 10). However, the factors grouped in this category and their associated variability differed greatly among the indices. Another important general source of uncertainty is the residual variance of the model, which accounted for an average of 29.2±9.5% (in mean±SE; see Table 11, Fig. 10) of the total variability among the different indices. In contrast, our results show that neither the temporal scale of sampling nor the human-associated source of error are important sources of uncertainty when classifying the ecological status of water bodies, as indicated by the low proportion of the total variance explained by the factors “year” and "surveyor" in the indices in which they were measured (5.4±2.3% and 2±2% respectively in mean±SE; see Table 11, Fig. 10).

4. Discussion

Including uncertainty estimation into assessment schemes is a major challenge of the next phase of WFD implementation (Hering et al. 2010). Even though the underlying statistical principles are relatively simple and appropriate tools for uncertainty estimation are available (e.g. Clarke and Hering 2006, Carstensen 2007), data addressing the individual sources of error are still needed, such as temporal and spatial variation of sampling, as well as differences between surveyors. This study is one of the first ones in which uncertainty analyses have been applied to several marine macrophyte based indexes, bringing some light to adequate designs in order to assess the ecological status of water bodies. Our results reveal that when analysing macrophyte communities, the factors related to the spatial scale of sampling added the highest levels of uncertainty whilst temporal variation and variability among surveyors were low. In addition, the residual term of the analysis added relatively high levels of uncertainty to the water body status classification of most indices, indicating that there are still unknown sources of variability that must be captured within the monitoring programmes.

Spatial variability has always been observed in natural communities, which becomes an important constrain when up scaling natural processes (Landres et al. 1999). In this study, the high levels of uncertainty associated to this factor are not surprising and may be related to the already observed high horizontal and vertical heterogeneity displayed by macrophyte communities (Ballesteros et al. 2007). Vertical variability has been attributed to factors associated to light attenuation with depth (Duarte 1991) and to the low rates of herbivory in deep sites compared to shallow depths (Tomas et al. 2005, Prado et al. 2007, Korpinen et al. 2007). All those natural processes, independent of any anthropogenic disturbances, influence structural and physiological parameters of macrophyte communities (Martínez-Crego et al. 2008), for which sampling at multiple depths result in highly variable EQR scores (from 25% to 37% of total variance in POMI and EI respectively). To reduce the risk of misclassification when assessing the ecological status of macrophyte communities, a relatively easy solution is that depth should remain fixed or be controlled in monitoring programs (see also Bennett et al. 2011). On the other hand, horizontal variability has been attributed to several factors acting from local (i.e. nutrient availability, sediment redox potential; Alcoverro et al. 1995) to regional scales (i.e. light, temperature; Marbà et al. 1996) that again influence structural and physiological parameters (Martínez-Crego et al. 2008). To capture this horizontal heterogeneity, bio-monitoring programmes must include sampling at different spatial scales, providing robust estimates of the ecological quality status classification at the water body level that include as much of this variability as possible, thereby minimizing the risk of misclassification (Kelly et al. 2009, Bennett et al. 2011). Even though bio-monitoring programmes from the different indices include sampling at several sites within each water body, only few of them include additional scales of replication below this level (POMI and SQI), resulting in a generally high uncertainty associated to the "site" factor (MSMDI, EDL, EI, RICQI). In these indices, it is strongly recommended to increase the sampling effort by adding a larger number of sites and within them, collecting different samples and averaging the metric values to provide robust estimates and minimize their associated risk of misclassification. This greater sampling effort may substantially increase the time and expense of the monitoring programmes, although it can be partially solved by maintaining the same number of replicates but just modifying the spatial sampling design to achieve a balance between financial constrains and a desirable index reliability. At a broader spatial scale, high variability among regions may indicate that they are separating groups of water bodies of similar ecological quality status. However, since this variability is above the scale of water body, at which the quality status is measured in the WFD, the risk of misclassification does not need to be minimized but included in the model and take into consideration when interpreting the uncertainty analysis results.

For the remaining factors, the uncertainty surrounding estimates in ecological status classification was very low within water bodies. Especially surprising is the case of inter-annual variability, which represented only between 1% and 9.7% of total variance depending on the index. As also reported by Bennett et al. (2011), this signifies that the EQR scores of water bodies are fairly consistent throughout the years, for which the frequency of sampling could be increased without greatly reducing the precision of ecological status estimates. Also surprising is the low variability among surveyors, which accounted only from 0% to 4% of total variance (for POMI and MSDMI respectively). This may be attributed to the fact that these particular macrophyte-based indices do not require complicated taxonomic identifications, which can greatly affect the precision of the EQR estimations in the case of other classification methods based on diatoms (Prygiel et al. 2002, Kelly et al. 2009) or freshwater macrophyte communities (Staniszewski et al. 2006). Finally, the residual term of the analysis represents all the variance that cannot be attributed to any of the factors included in the model, giving an idea of the accuracy of our approximation. In our study, it represented a relatively large proportion of total variance among the different indices (29.2±9.5% in mean±SE), indicating that other unknown sources of uncertainty may be affecting the ecological status classification of water bodies. In order to keep this variance to the minimum, further data concerning other factors related to the sampling design may need to be collected in those indices where it is relatively large (spatial variance, temporal variance, variance among surveyors, etc.).

Furthermore, our results showed that the risk of misclassifying the quality status of water bodies is also affected by the width of the status class in which the EQR score falls, as reported in Kelly et al. (2009), with narrower classes leading to greater probabilities of misclassification. Thus, indices in which the EQR range is not equally split into the 5 official classes present, for a certain variance associated to a factor, different uncertainty levels depending on the status class (see EDL, POMI, RICQI and EEI-c). This fact have drastic implications for bio-monitoring programs, because a greater sampling effort may need to be assigned to water bodies whose EQR score falls within the narrower status classes in order to reduce their associated variability and increase the confidence of the classification.

5. Conclusions

In summary, our study confirmed that the analysis of the uncertainty associated to the ecological quality status classification of water bodies are a good proxy to identify and quantify the factors that may affect the risk of misclassification. When applied to macrophyte monitoring programs, we have observed that the main sources of uncertainty are mostly associated to the sampling spatial scales, while temporal or human-induced errors seem to be less relevant. As a guide for taking management decisions, adequate sampling designs that include replication at different spatial scales within water bodies may substantially reduce this uncertainty. In some cases, it is not increasing the sampling effort but distributing it more efficiently within the allocated time and budget constrains that we will be able to maximize the confidence of estimations when assessing ecosystem health under the WFD.

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Figure 1: Map of the locations where bio-monitoring data from the different indices was obtained.

Figure 2: Probability of misclassifying the ecological status class associated to the different factors analysed for MSMDI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.

Figure 3: Probability of misclassifying the ecological status class associated to the different factors analysed for EDL. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.25; Poor = 0.26 – 0.5; Moderate = 0.51 – 0.74; Good = 0.75-0.9 and High = 0.91 – 1.

Figure 4: Probability of misclassifying the ecological status class associated to the different factors analysed for POMI. Vertical dashed lines represent the boundaries

of each status class. Bad = EQR values from 0 – 0.09; Poor = 0.1 – 0.324; Moderate = 0.325 – 0.549; Good = 0.550-0.774 and High = 0.775 – 1.

Figure 5: Probability of misclassifying the ecological status class associated to the different factors analysed for CYMOX. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.

Figure 6: Probability of misclassifying the ecological status class associated to the different factors analysed for RICQI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.82 and High = 0.83 – 1.

Figure 7: Probability of misclassifying the ecological status class associated to the different factors analysed for EEI-c. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.

Figure 8: Probability of misclassifying the ecological status class associated to the different factors analysed for EI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.

Figure 9: Probability of misclassifying the ecological status class associated to the different factors analysed for SQI. Vertical dashed lines represent the boundaries of each status class. Bad = EQR values from 0 – 0.2; Poor = 0.21 – 0.4; Moderate = 0.41 – 0.6; Good = 0.61-0.8 and High = 0.81 – 1.

Table 1. Macrophyte-based classification indices included in this study with their main characteristics.

Index	Region of application	Target species	Metric/s used	Status Class Boundaries	References
MSMDI Multi Species Maximum Depth Index	North Sea (Norway) Coastal Waters	*Saccharina latissima* *Chondrus crispus* *Rhodomela confervoides* *Coccotylus truncata* *Phyllophora pseudoceranoides* *Halidrys siliquosa* *Delesseria sanguinea* *Phycodrys rubens* *Furcellaria lumbricalis*	Lower depth limit	0.2 / 0.4 / 0.6 / 0.8	Swedish Environmental Protection Agency, 2007
EDL Eelgrass Depth Limit	Baltic Sea (Denmark) Coastal Waters	*Zostera marina*	Lower depth limit	0.25 / 0.5 / 0.74 / 0.9	Krause-Jensen et al., 2005
POMI *Posidonia oceanica* Multivariate Index	Western Mediterranean (Spain and Croatia) Coastal Waters	*Posidonia oceanica*	Physiological, morphological, population (density) and community, integrated onto a single scale using Principal Component Analysis	0.1 / 0.325 / 0.55 / 0.775	Romero et al., 2007
CYMOX *Cymodocea nodosa* Multi-bioindicator Index	Ebro Delta Bays - Western Mediterranean (Spain) Transitional Waters	*Cymodocea nodosa*	Physiological, morphological, population (density) and community, integrated onto a single scale using Principal Component Analysis	0.2 / 0.4 / 0.6 / 0.8	Oliva et al., *in press*
RICQI Rocky Intertidal Community Quality Index	North Atlantic (Spain) Coastal Waters			0.2 / 0.4 / 0.6 / 0.82	Díez et al., 2012
EEI-c Ecological Evaluation Index	Lesina Lagoon - Adriatic Sea (Italy) Transitional Waters	*Cymodocea nodosa*-ESG IA *Ruppia cirrhosa*-ESG IA *Cystoseira barbata*-ESG IB *Gracilaria bursa-pastoris*-ESG IIA *Cladophora* spp.-ESG IIB *Ulva* spp.-ESG IIB	Coverage (%) of 5 different Ecological Status Groups clustered hierarchically into two ESG's	0.04 / 0.25 / 0.48 / 0.76	Orfanidis et al., 2011
EI Ecological Index	Varna Bay - Black Sea (Bulgaria) Transitional Waters	*Cystoseira barbata*-ESGI *Cystoseira crinite*- ESGI *Corallina* spp.- ESGI *Gelidium latifolium*- ESGI	Biomass proportion (%) of different macrophyte species classified in 2 different Ecological Status Groups:	0.2 / 0.4 / 0.6 / 0.8	Dencheva *in press*

		Zostera noltii- ESGI *Zostera marina*- ESGI *Potamogeton pectinatus*- ESGII *Ulva* spp.- ESGII *Cladophora* spp.- ESGII *Ceramium* spp.- ESGII *Chaetomorpha* spp.- ESGII *Polysiphonia* spp.- ESGII	sensitive (ESGI) and tolerant (ESGII)		
SQI Seagrass Quality Index	Mondego Bay (Portugal) Transitional Waters	*Zostera noltii*	- Taxonomic Composition (TC) - Bed Extent (BE) - Shoot Density (SD)	0.2 / 0.4 / 0.6 / 0.8	

Table 2. Factors of the different groups included in the main sources of uncertainty, and the variance components.

Index	Main sources of uncertainty			Variance components
	Temporal scale	Spatial scale	Human-associated error	
MSMDI Multi Species Maximum Depth Index	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si})	· Surveyor (σ^2_{Su})	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_{Sur} + \sigma^2_R$
EDL Eelgrass Depth Limit	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_R$
POMI *Posidonia oceanica* Multivariate Index	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si}) · Zone:(Site:Water Body:Region) (σ^2_Z) · Depth (σ^2_D)	· Surveyor (σ^2_{Su})	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_Z + \sigma^2_D + \sigma^2_{Su} + \sigma^2_R$
CYMOX *Cymodocea nodosa* Multi-bioindicator Index	· Year (σ^2_Y)	· Region (σ^2_{Rg}) · Water Body:Region (σ^2_{WB}) · Site:(Water Body:Region) (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Rg} + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_R$
RICQI Rocky Intertidal Community Quality Index	· Year (σ^2_Y)	·Site:Water Body (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Si} + \sigma^2_R$
EEI-c Ecological Evaluation Index	-	· Water Body (σ^2_{WB}) · Site:Water Body (σ^2_{Si})	-	$\sigma^2_T = \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_R$
EI Ecological Index	· Year (σ^2_Y)	· Water Body (σ^2_{WB}) · Site:Water Body (σ^2_{Si}) · Depth (σ^2_D)	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{WB} + \sigma^2_{Si} + \sigma^2_D + \sigma^2_R$
SQI Seagrass Quality Index	· Year (σ^2_Y)	· Site (σ^2_{Si}) · Zone:Site (σ^2_Z) · Sample:(Zone:Site) (σ^2_{Sa})	-	$\sigma^2_T = \sigma^2_Y + \sigma^2_{Si} + \sigma^2_Z + \sigma^2_{Sa} + \sigma^2_R$

Table 3. MSMDI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of five random effects. Colon between factors represents nesting (i.e. Site:WB signifies that site is nested within water body).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	21	Crossed	0.016156	0.000261	2
Region	(Intercept)	2	Crossed	0.000000	0.000000	0
Water Body	(Intercept)	12	Crossed	0.071020	0.005044	42
Site:WB	(Intercept)	20	Nested	0.054312	0.002950	24
Surveyor	(Intercept)	4	Crossed	0.021960	0.000482	4
Residual				0.057723	0.003332	28

Table 4. EDL results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	23	Crossed	0.088461	0.007825	10
Region	(Intercept)	9	Crossed	0.155929	0.024314	30
Water Body:Region	(Intercept)	20	Nested	0.068029	0.004628	6
Site:(WB:Region)	(Intercept)	254	Nested	0.139132	0.019358	24
Residual				0.156419	0.024467	30

Table 5. POMI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of seven random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	6	Crossed	0.050508	0.002551	5
Region	(Intercept)	3	Crossed	0.125150	0.015663	30
Water Body:Region	(Intercept)	50	Nested	0.088485	0.007830	15
Site:(WB:Region)	(Intercept)	103	Nested	0.048587	0.002361	4
Zone:(Site:WB:Region)	(Intercept)	119	Nested	0.039436	0.001555	3
Depth	(Intercept)	2	Crossed	0.116480	0.013568	26
Surveyor	(Intercept)	4	Crossed	0.000001	0.000000	0
Residual				0.094870	0.009000	17

Table 6. CYMOX results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	4	Crossed	0.000000	0.000000	0
Region	(Intercept)	2	Crossed	0.000005	0.000000	0
Water Body:Region	(Intercept)	6	Nested	0.240210	0.057701	76
Site:(WB:Region)	(Intercept)	14	Nested	0.041205	0.001698	2
Residual				0.126980	0.016124	21

Table 7. RICQI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Site:(WB:Region) signifies that site is nested within water body that, at the same time, is nested within region).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	3	Crossed	0.073332	0.005378	14
Site	(Intercept)	7	Crossed	0.164836	0.027171	73
Residual				0.068418	0.004681	13

Table 8. EEI-c results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of three random effects. Colon between factors represents nesting (i.e. Replicate:(Site:WB) signifies that replicate is nested within site that, at the same time, is nested within WB).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Water Body	(Intercept)	4	Crossed	0.292036	0.085285	68
Site:WB	(Intercept)	6	Nested	0.000006	0.000000	0
Replicate:(Site:WB)	(Intercept)	18	Nested	0.086976	0.007565	6
Residual				0.179911	0.032368	26

Table 9. EI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Site:WB signifies that site is nested within water body).

Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}
Year	(Intercept)	6	Crossed	0.050570	0.002557	1
Water Body	(Intercept)	10	Crossed	0.250052	0.062526	34
Site:WB	(Intercept)	19	Nested	0.215546	0.046460	25
Depth	(Intercept)	5	Crossed	0.259052	0.067108	37
Residual				0.071688	0.005139	3

Table 8. SQI results of linear mixed effects model fit by restricted maximum likelihood (REML). Untransformed EQR scores analysed as a function of four random effects. Colon between factors represents nesting (i.e. Zone:Site signifies that zone is nested within site).

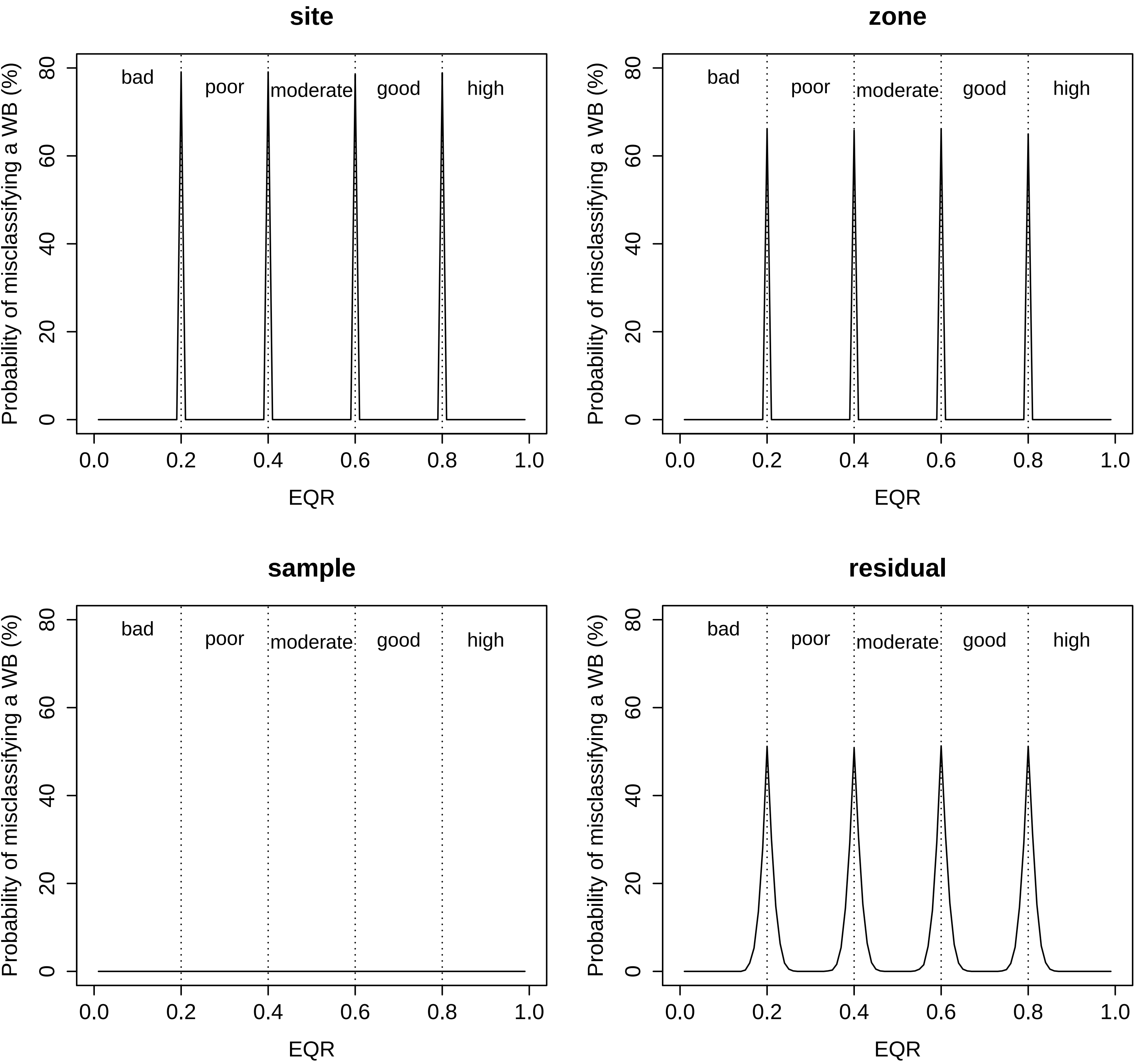
Groups	Name	Levels	Type	St. Dev.	Variance	P_{samp}

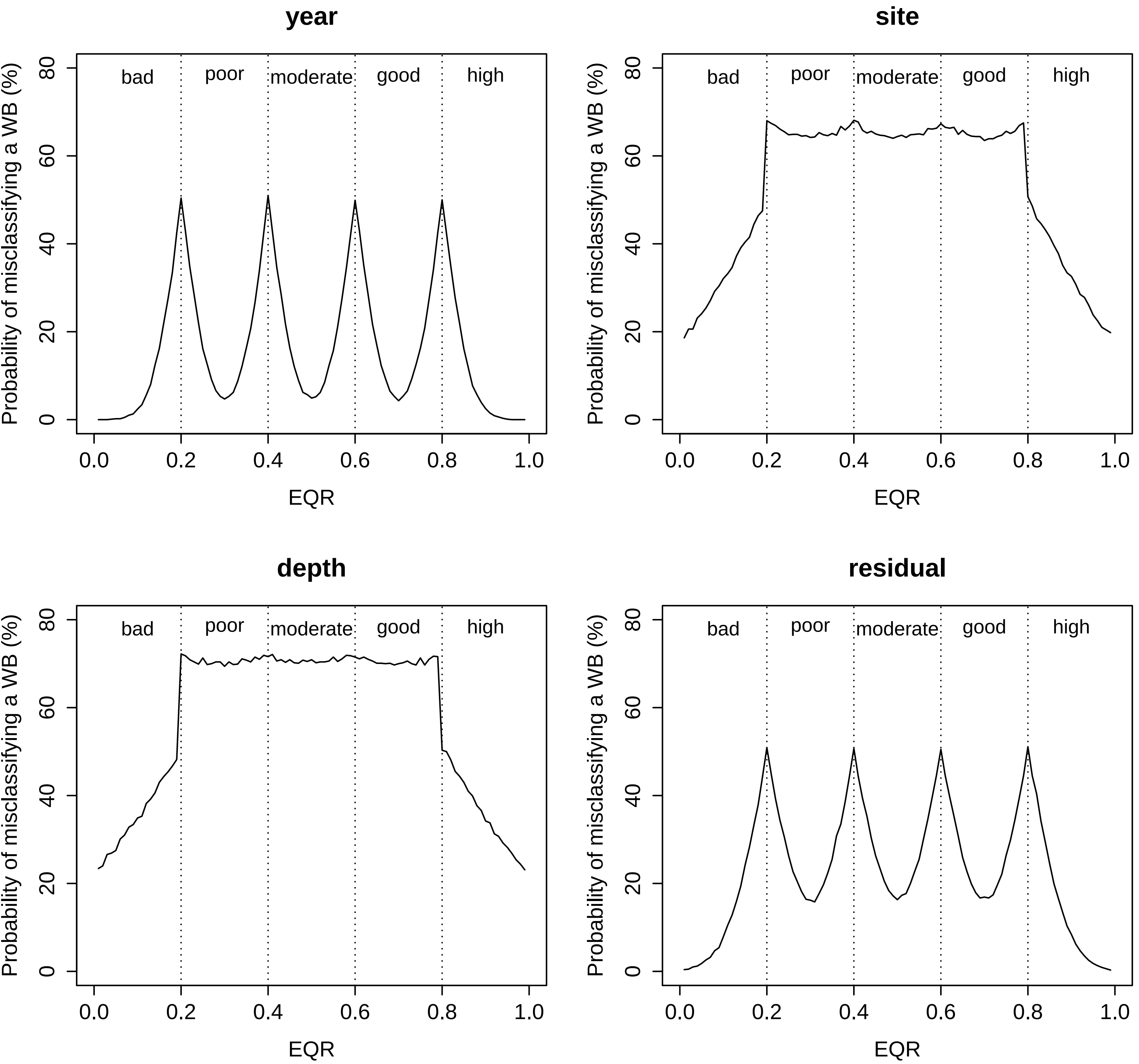
Site	(Intercept)	2		Crossed	0.024843	0.000617	3
Zone:Site	(Intercept)	4		Nested	0.034845	0.001214	6
Sample:(Zone:Site)	(Intercept)	8		Nested	0.000000	0.000000	0
Residual					0.139830	0.019054	91

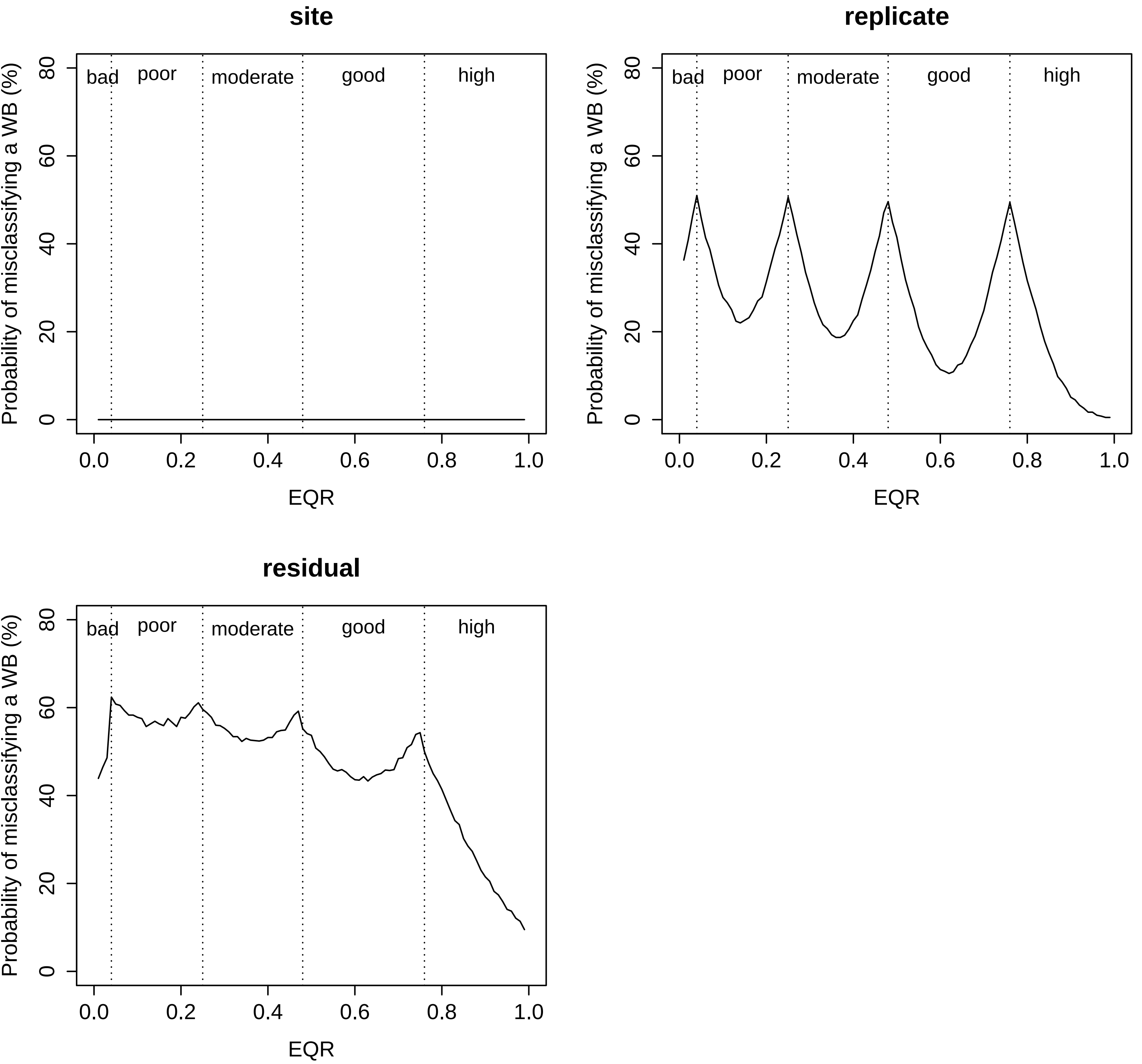
Table 9. Proportion of the total variance (in %) explained by the different factors grouped in the main sources of uncertainty for each index, excluding WB.

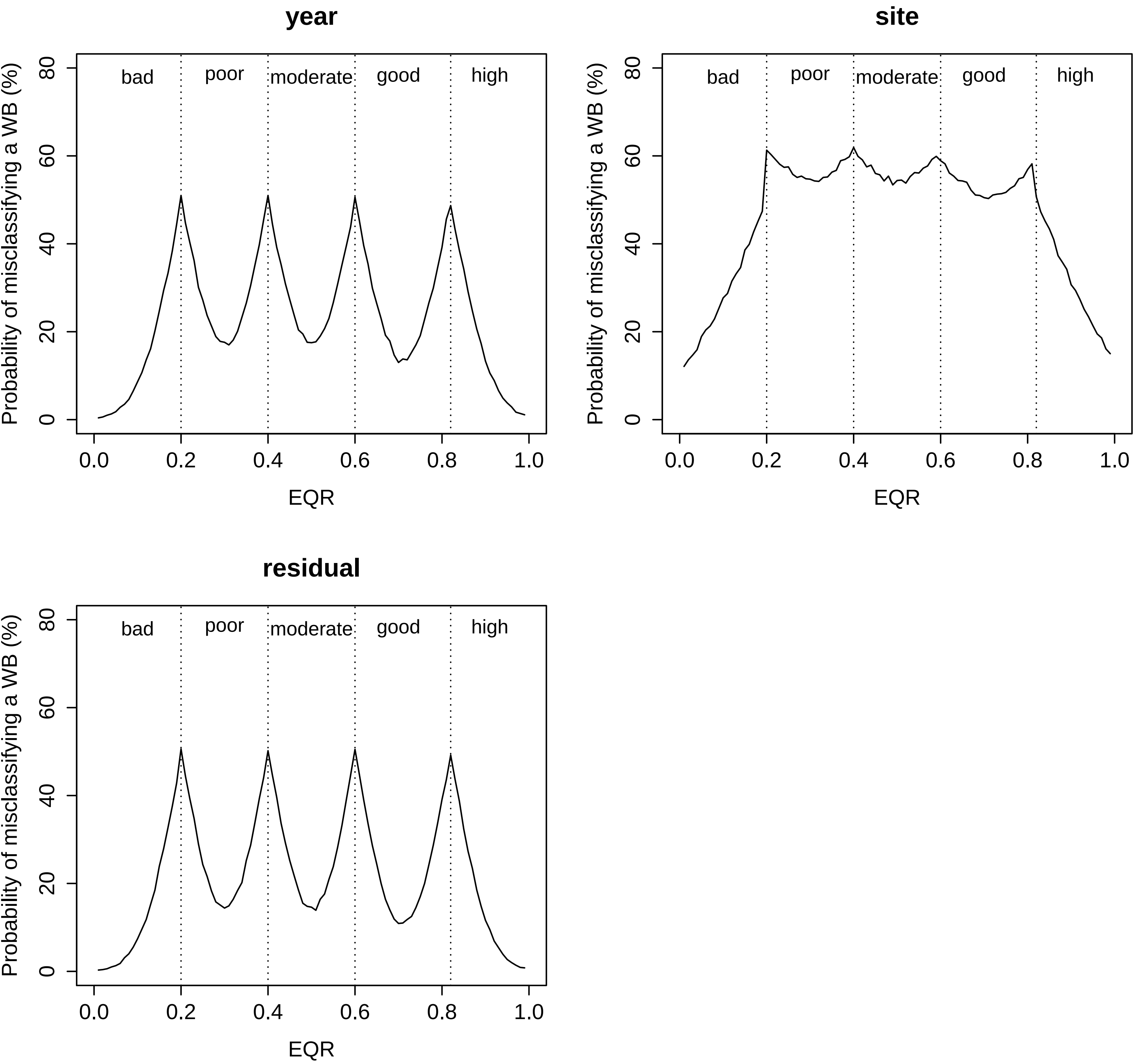
Index	

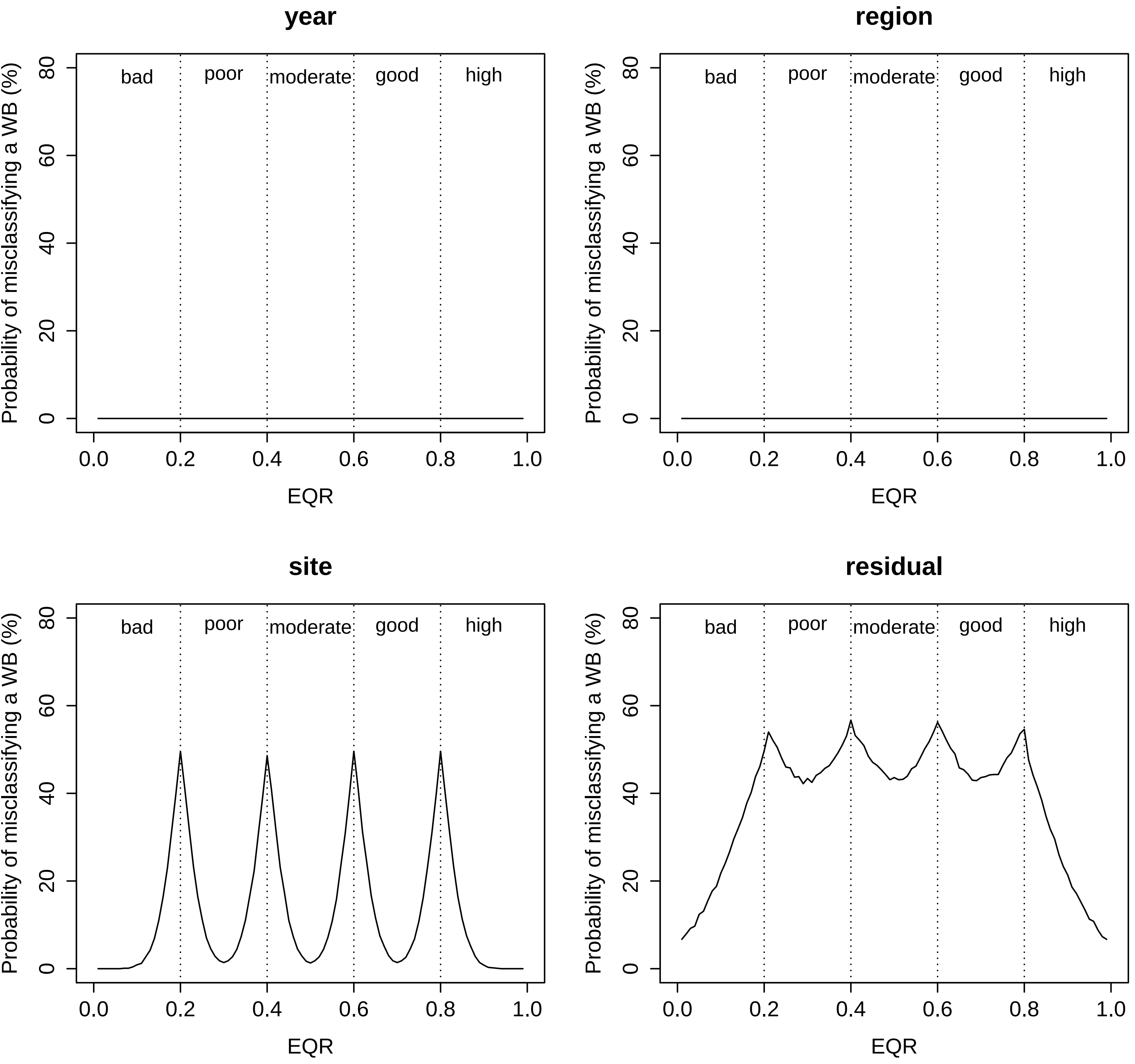
	of uncertainty			
	Temporal scale	Spatial scale	Human-associated error	Residual
MSMDI Multi Species Maximum Depth Index	2	24	4	28
EDL Eelgrass Depth Limit	10	54	-	30
POMI *Posidonia oceanica* Multivariate Index	5	63	0	17
CYMOX *Cymodocea nodosa* Multi-bioindicator Index	0	2	-	21
RICQI Rocky Intertidal Community Quality Index	14	73	-	13
EEI-c Ecological Evaluation Index	-	0	-	30
EI Ecological Index	1	62	-	3
SQI Seagrass Quality Index	-	9	-	91
mean	5	36	2	29
SE	2	11	2	9

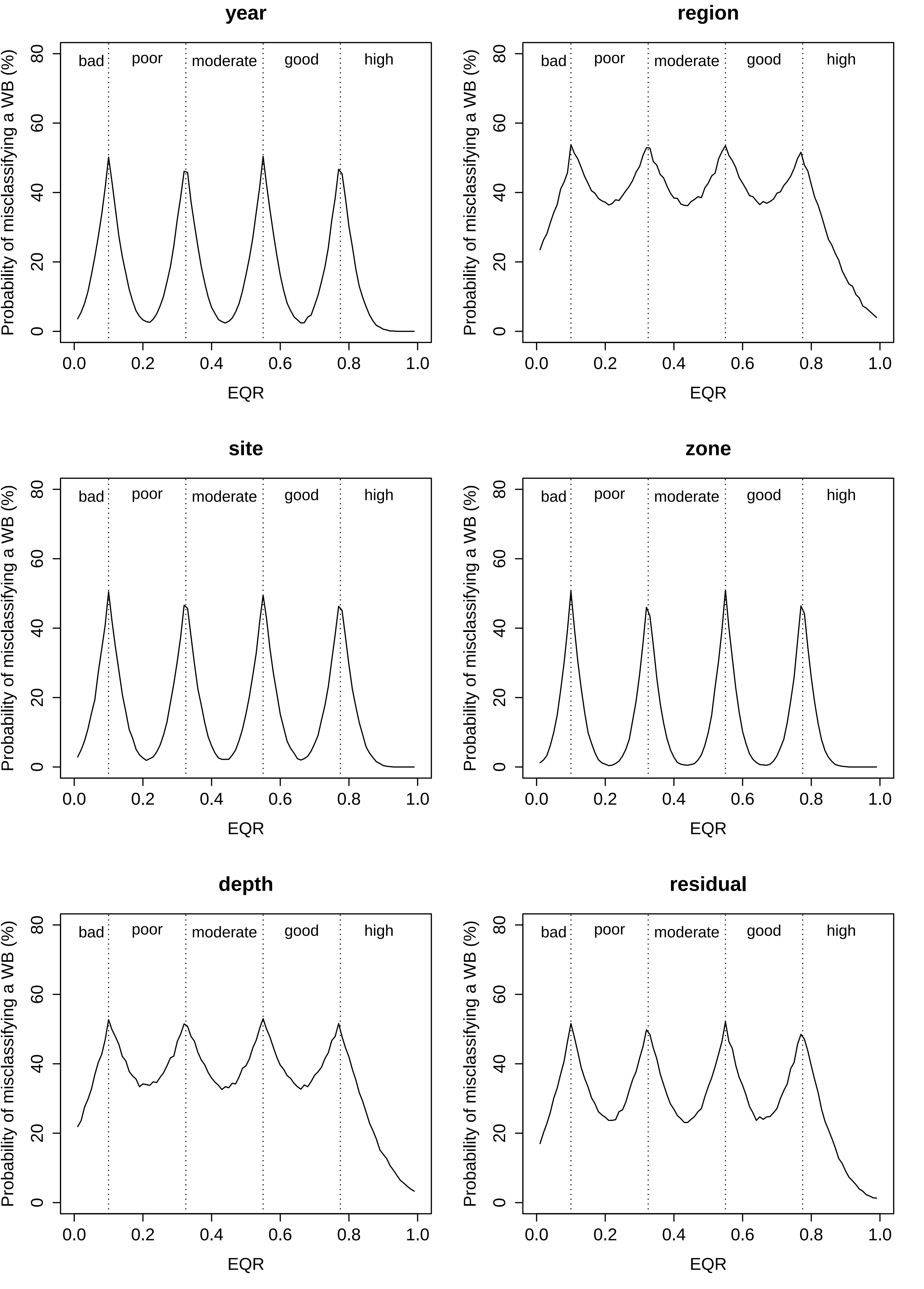

site
zone
sample
residual
bad
poor
moderate
good
high
Probability of misclassifying a WB (%)
EQR
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0

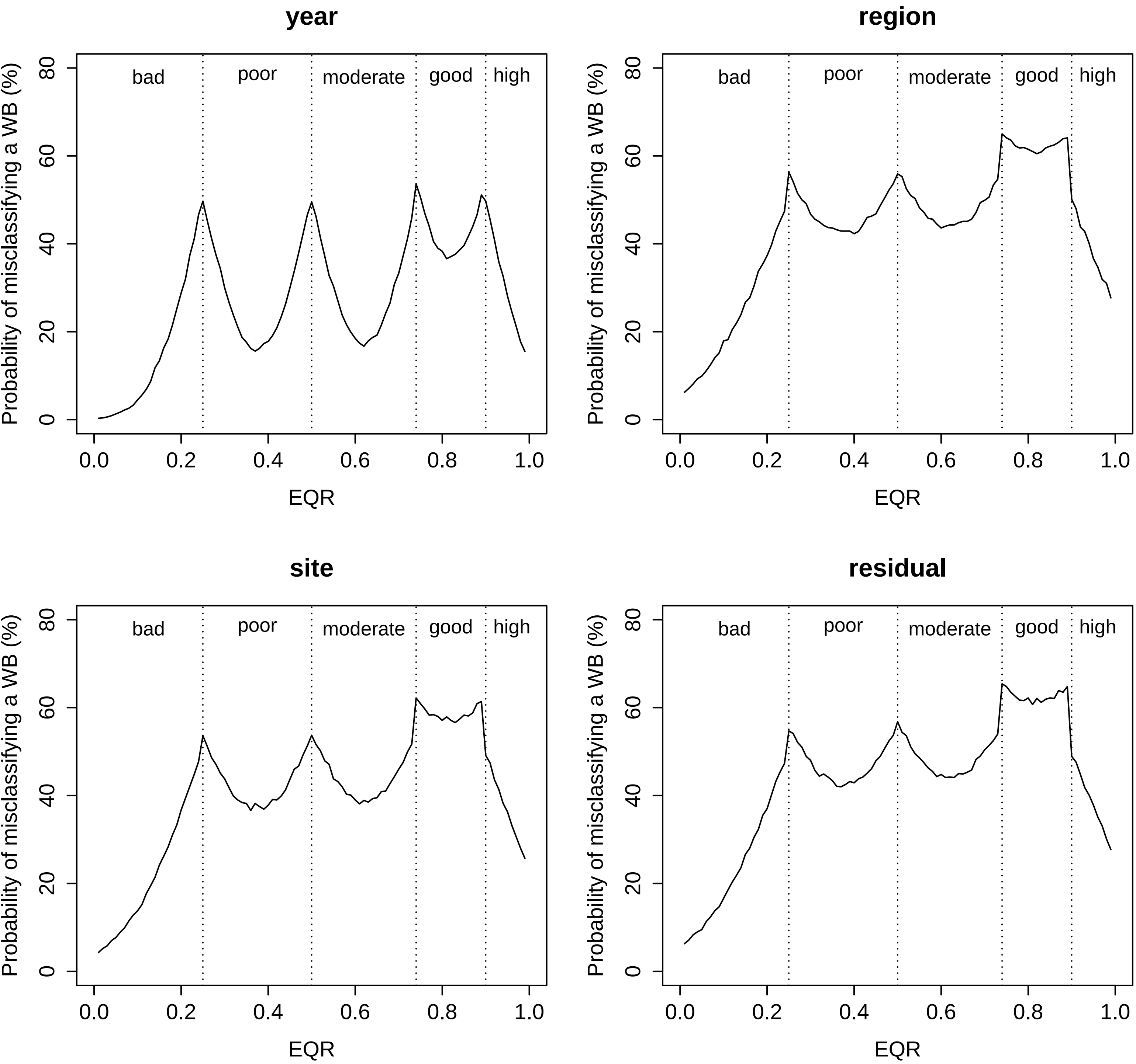

year
site
depth
residual
bad
poor
moderate
good
high
Probability of misclassifying a WB (%)
EQR
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0

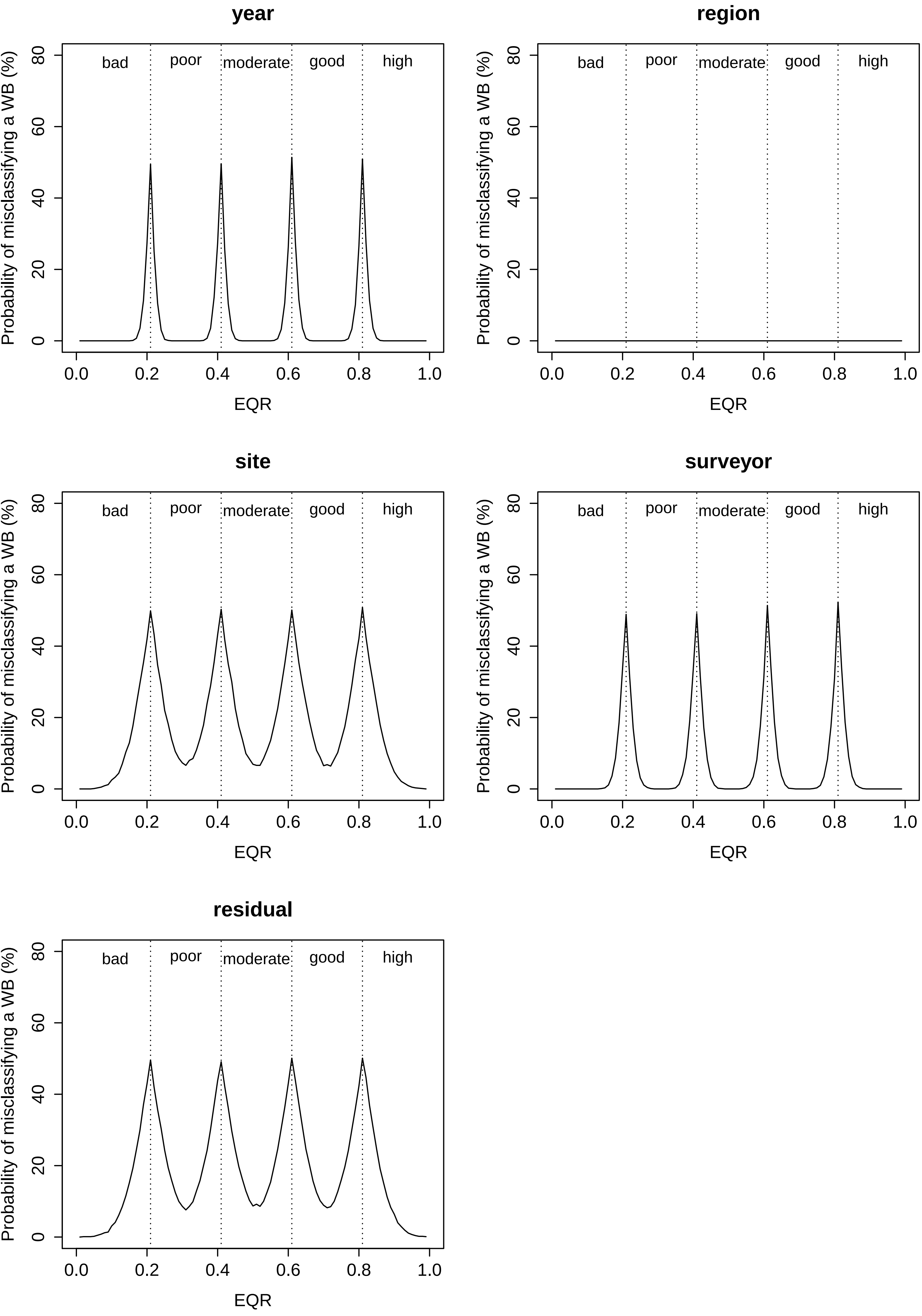

site
replicate
residual
bad
poor
moderate
good
high
Probability of misclassifying a WB (%)
EQR
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0


year
site
residual
bad
poor
moderate
good
high
Probability of misclassifying a WB (%)
EQR
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0


year
region
site
residual
Probability of misclassifying a WB (%)
EQR
bad
poor
moderate
good
high
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0


year
region
site
zone
depth
residual
bad
poor
moderate
good
high
Probability of misclassifying a WB (%)
EQR
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0


year
region
site
residual
Probability of misclassifying a WB (%)
EQR
bad
poor
moderate
good
high
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0


year
region
site
surveyor
residual
bad
poor
moderate
good
high
Probability of misclassifying a WB (%)
0
20
40
60
80
EQR
0.0
0.2
0.4
0.6
0.8
1.0