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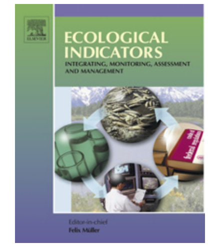

Comparison of two methods for quality assessment of macroalgae assemblages, under different pollution types

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ABSTRACT

The selection of adequate methodologies for the assessment of different biological quality elements is urgently needed for the application of the water framework directive (WFD 2000/60/EEC). In the case of macroalgae in coastal waters of the North East Atlantic, two methodologies have been proposed: the reduced species list (RSL) index and the quality of rocky bottoms (CFR) index. Both methods use multimetric approaches to evaluate the quality of macroalgae assemblages, which are based on community characteristics (species/populations richness, cover, percentage of opportunistic species, ecological state groups ratio, etc.). In this paper the results of applying both indices on three different types of pollution gradients in the North coast of Spain (bay of Biscay) are presented, in order to test their usefulness and intercalibration possibilities. In general terms, the CFR index responded more accurately than the RSL index to the pollution gradients under study. With respect to the indicators used in the current evaluation, richness, opportunistic species and cover seemed to be the most accurate for quality assessment of macroalgal communities. While the first two indicators are taken into account in both indices, the latter (cover) is only considered in the CFR index, even though the abundance of macroalgae is one of the aspects to be included in the evaluation of this biological element, according to the WFD.

1. Introduction

According to the water framework directive (WFD 2000/60/EEC) macroalgae are one of the biological quality elements to be evaluated for the assessment of the ecological status of coastal water bodies. Recently, several methodologies have been proposed to accomplish this task e.g. the ecological evaluation index (EEI) (Orfanidis et al., 2001), the reduced species list index (RSL) (Wells, 2004; Wells et al., 2007), the littoral cartography methodology (CARLIT) (Ballesteros et al., 2007) or the quality of rocky bottoms index (CFR) (Juanes et al., 2008). The advantages and disadvantages of these methods have been analysed by Juanes et al. (2008).

In general terms, the conceptual basis of all these approaches consists of an analysis of the relative abundance of pollution sensitive or indicator species. In an increasing pollution gradient it is expected that the most sensitive taxa, generally the most specialized or k-selected species, are gradually replaced by pollution tolerant and indicator species, typically opportunistic or r-selected species. In the case of the EEI and the RSL indices, the classification of species is based on the morphological and functional-form groups described by Littler and Littler (1980, 1984) and subsequently adapted by Orfanidis et al. (2001) to divide species into two ecological state groups (ESG): the ESG I, including species with a thick or calcareous thallus, low growth rates and long life cycles (perennials), and the ESG II, including sheet-like and

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filamentous species with high growth rates and short life cycles (annuals). However, as stated by Arévalo et al. (2007), the functional-form group hypothesis was originally proposed to predict productivity and other ecological attributes (e.g. grazing resistance, competitive abilities, reproductive effort), but not resistance to pollution.

Thus, in the recent literature on this topic misleading interpretations of the sensitivity levels assigned to the same species are found, as observed in the case of the different quality values assigned to *Corallina* spp. populations by Orfanidis et al. (2001) and Ballesteros et al. (2007). While the first authors considered *Corallina* as a late-successional species (ESG I), which would represent a high ecological quality, the second authors assigned this species to an intermediate-low sensitivity level hence its presence would indicate a moderate ecological quality. So, it seems clear that the development of suitable and accurate indices based on macroalgae as pollution indicators requires, first of all, a consensus about the pollution sensitivity level assigned to each macroalgae species. Similar inconsistencies were found by Puente et al. (2008) when assigning macroinvertebrate species to different sensitive/tolerant groups in estuarine areas.

Before the definitive acceptance of the metrics to be used for the quality assessment of each biological quality element, the WFD demands that member states undertake an intercalibration process among all the proposed metrics. In the case of the North East Atlantic geographical intercalibration group (NEA GIG) two different tools have been proposed for the assessment of macroalgae, the RSL index (Wells, 2004; Wells et al., 2007) and the CFR index (Juanes et al., 2008). The most important difference between these indices is the exclusive use of macroalgae abundance estimates (cover) by the CFR index, which follows the basic requirements of the WFD. Another difference between the indices is that the CFR index is suitable for both intertidal and subtidal areas, while the RSL is only applicable to the former.

On the other hand, both indices include the opportunistic species and the richness among their indicators, though they differ in their way of application. Thus, in the CFR index there is not an exhaustive analysis of macroalgae at the species level, instead only characteristic macroalgae populations with a noticeable presence (>1% cover) are used for the richness estimates. Other studies have also suggested that monitoring efforts should be directed towards perennial species with a sufficient depth distribution (Eriksson and Bergström, 2005), considering that ephemeral algae are probably more stochastic in their occurrence than perennial algae. Regarding the proportion of opportunistic species, the RSL index considers the relative number of opportunistic species in relation with total macroalgal richness, while the CFR index evaluates their relative cover in respect to the total cover.

Another basic characteristic of these methodologies is that they are relatively easy to apply and have an effective cost–benefit relation, so they can be scientifically rigorous and at the same time, useful tools to carry out extensive management works. In this sense, both indices use non-destructive data collection methods but their main difference lies on the way in which the scoring system is applied. While the RSL index requires the identification of all macroalgae species present in a reduced species list for posterior analysis and score assignation of each indicator, the CFR index is designed for its direct application in situ through ranges based scoring system of each indicator. One of the reasons for this simplification in the CFR index is that it was also designed for its application in extensive subtidal areas by SCUBA diving or by remotely operated vehicles (ROVs), which requires a simplified assessment methodology.

Given that the development and selection of evaluation indices is a fundamental task to assess the ecological status of coastal water bodies, the aim of this paper is to test and validate the suitability of the CFR and the RSL indices to monitor water quality by examining intertidal macroalgae communities. In order to analyse the possibilities and requirements for the intercalibration of both methods, the capability of each method to adequately detect and quantify differences in the quality of coastal macroalgae communities along different types of pollution gradients was studied at both the global index (CFR and RSL) and the single indicator levels (richness, cover, etc). The degree of adjustment of the quality assessment results obtained by the two methods was then statistically analysed.

2. Methodology

2.1. Study area

The experimental design was carried out in the summer 2006 at three places located along the coast of Cantabria (N Spain), each one exposed to different types of urban and industrial discharges (Fig. 1). The first site, Liñera, is located near a secondary-treatment urban waste-water plant for about 15,000 inhabitant equivalents, discharging directly on the coastline. The high concentrations of total nitrogen (24 mgN/l), total phosphorous (3 mgP/l), Biological oxygen demand (28 mgO/l) and total suspended solids (63 mg/l) (average values of unpublished data from the environmental department of the Government of Cantabria), produce turbidity and oxygen demand in the surrounding coastal area, but especially an increment of the natural levels of nutrients with the resulting eutrophication risk. At the second place, Usgo, a high density inert industrial effluent of a sodium carbonate factory, mainly composed of $CaCl_2$ (TSS:23 g/l and 1400 m^3/h flow), with 65 °C and pH 11, has been discharged for 40 years directly on the coastline, and since 2002 through a submarine outfall (−15 m depth) (Revilla et al., 2007). Siltation, turbidity and abrasive effects in this coastal area are easily recognized. The third site, Ontón, is located near the industrial effluent of a fluoride factory which discharges about 11.5 t/year of fluorides directly on the coastline (MMA, 2001), with an average concentration of 29.7 mg/l and point measured values of pH 11.75 (unpublished data from the environmental department of the Government of Cantabria).

The intertidal zonation pattern of the macroalgae communities along the Cantabrian coast can be divided into two main fringes; the mid-littoral (dominated by *Corallina* spp. and accompanied by Calcareous encrusters, *Caulacanthus ustulatus*, *Ceramium* spp., *Chondracanthus* spp., *Osmundea* spp., etc...) and the infralittoral (dominated by *Bifurcaria* spp. and accompanied by *Stypocaulon scoparia*, *Codium* spp., *Cladostephus* spp.,

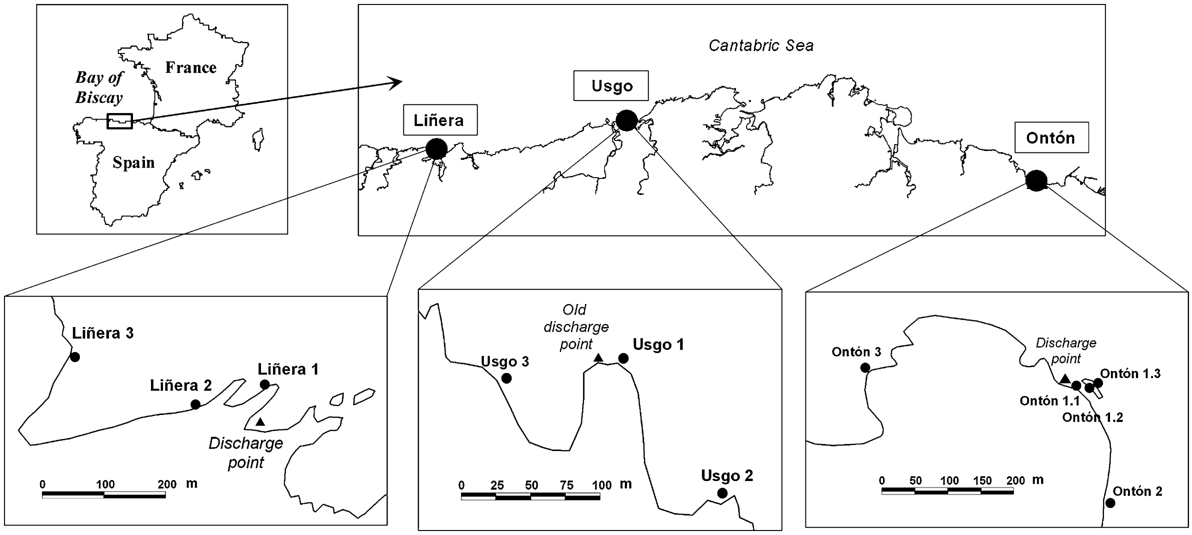

Fig. 1 – Location of the stations (●) and the discharge points (▲) at each of the studied sites.

various red small folioses, Champiaceae, etc....). Other important communities developing between the infralittoral and the shallow subtidal area are those of *Gelidium* spp. and *Cystoseira* spp. (CHN, 1998a, 1998b; Puente, 2000; UC-GC, 2005).

The communities found in the control stations located furthest away from the analysed discharge points respond to these zonation patterns. However, the composition of macroalgae assemblages at the stations located closer to the discharge points show clear signs of alterations. In the case of Liñera, the great development of macroalgae coverage and diversity, but specially the elevated proportion of opportunistic species (*Ulva* sp., *Cladophora* sp., *Ceramium* sp.) respond to the discharge of nutrient enriched waters, causing some degree of eutrophication in the surrounding area. In the case of Usgo, the continuous discharge of great amounts of suspended solids, with the consequent turbidity and erosive effects, has produced a severe reduction of macroalgae coverage near to the old discharge point. However, the relocation of the discharge point from the intertidal to 15 m depth, has allowed an incipient recolonisation of the most affected areas by both opportunistic and non-opportunistic species. Finally, the toxic effects of the discharge in Ontón produced not only a severe reduction of both coverage and diversity of macroalgae, but also a visible depigmentation of the few characteristic macroalgae found in the stations located near the discharge point.

2.2. Experimental design

Two methodological approaches, the RSL index and the CFR index, were applied for the quality assessment of macroalgae assemblages, at several exposed and semi-exposed intertidal stations (3–5) located along the pollution gradients of the three mentioned contaminant sources. These pollution gradients are not analysed in a quantitative way, but in a qualitative way assuming that there are contaminant concentration gradients associated to the distance from the discharge points.

To compare the obtained and the expected results, the quality category of each station was estimated ‘‘a priori’’ based on expert judgement, according to their situation along the pollution gradient and depending on the apparent quality of the macroalgae communities. The application of this criterion presents serious limitations due to its relative subjectivity, and so, although it can be a valid approach to achieve the proposed objectives, the obtained results must be taken with care. For statistical purposes, a quantitative value was assigned to each quality category following a 1 (bad) to 5 (high) scale. In case of doubt between two categories, the assigned value corresponded to the average value, in order to perform correlation analyses, or to the higher values, in order to perform weighted kappa analyses.

The CFR index was applied in situ to each station, considering the intertidal macroalgae lists and the intertidal scoring criteria established for each of the four indicators that compose this index (coverage, richness, opportunistics and physiological state), according to Juanes et al. (2008).

The RSL index (Wells, 2004; Wells et al., 2007) was applied according to the guidelines established in the Milestone six report for coastal NE Atlantic GIG (European Commission, 2006), which has proved to produce better results than other variations of this index in preliminary analyses carried out with data from the Northern coast of Spain (unpublished data). Four different alternatives have been tested for the application of this index. The first alternative, referred to as ‘‘RSL-external’’, constitutes the original proposal from the UK and has been applied by using the complete list of all the identified species, 81 in total, and the ecological state groups (ESG) as proposed by Wells (2002). The second alternative, referred to as ‘‘RSL-local’’, considers both a reduced species list established for the local characteristics of the Cantabrian coast and ESG values in agreement with the ecological conditions of this coastal area (Table 1).

The other two alternatives (RSL-ext-Q and RSL-loc-Q) constitute modifications of the former ones, which include a quantitative correction factor for the amount of opportunistic

Table 1 – Reduced species list established for the Cantabrian coast and corresponding ESGs proposed for the RSL-external and the RSL-local alternatives

Species	ESG for RSL-external	ESG for RSL-local	Opportunistic
Blidingia/Derbesia	2	2	Yes
Bryopsis plumosa	2	2	Yes
Chaetomorpha spp.	2	2	Yes
Cladophora spp.	2	2	Yes
Codium adhaerens		1	
Codium tomentosum–fragile	2	1	
Enteromorpha spp. (now *Ulva* spp.)	2	2	Yes
Ulva spp.	2	2	Yes
Bifurcaria bifurcata		1	
Cladostephus spongiosus–verticillatus	2	1	
Colpomenia spp./*Leathesia* spp.	2	2	
Cystoseira baccata		1	
Cystoseira tamariscifolia		1	
Dictyota dichotoma	2	2	
Ectocarpaceae/*Sphacelaria* spp.	2	2	Yes
Fucus spiralis	1	1	
Fucus vesiculosus	1	1	
Laminaria spp.	1	1	
Nemalion helminthoides	2	2	
Pelvetia canaliculata	1	1	
Ralfsia verrucosa		1	
Saccorhiza spp.	1	1	
Sargassum muticum		1	
Scytosiphon spp.	2	2	
Stypocaulon (*Halopteris*) *scoparia*	2	1	
Epiphytic filamentous[a] (G1)	2	2	Yes
Small folioses[b] (G2)	2	1	
Champiaceae[c] (G3)	2	2	
Calcareous encrusters[d] (G4)	1	1	
Asparagopsis armata	2	2	
Catenella caespitosa	2	2	Yes
Caulacanthus ustulatus		1	
Chondracanthus (Gigartina) acicularis	2	1	
Chondria coerulescens		1	
Chondrus crispus	2	1	
Corallina elongata–officinalis/Jania	1	1	
Falkenbergia/Trailliella	2	2	
Gelidium latifolium	2	1	
Gelidium pusillum	2	1	
Gelidium corneum (sesquipedale)	2	1	
Gigartina spp.	2	1	
Gymnogongrus spp.		1	
Halurus equisetifolius	2	1	
Hildenbrandia spp.	1	1	
Lithophyllum byssoides	1	1	
Mastocarpus stellatus	2	1	
Osmundea (*Laurencia*) spp.	2	1	
Peyssonnelia spp.	1	1	
Plocamium/Sphaerococcus	2	2	
Porphyra spp.	2	2	Yes
Pterosiphonia complanata		1	

The opportunistic character of some species is also indicated.

[a] Group 1 (Epiphytic filamentous): *Ceramium*, *Pleonosporium*, *Aglaothamnion*, *Callithamnion*, *Antithamnion*, *Antithamionella*, *Polysiphonia*, *Dasya*, *Pterosiphonia*.

[b] Group 2 (Small folioses): *Apoglossum*, *Hypoglossum*, *Acrosorium*, *Nytophyllum*, *Cryptopleura*, *Rhodophyllis*, *Stenogramme*, *Callophyllis*, *Kallymenia*, *Rhodymenia*.

[c] Group 3 (Champiaceae): *Champia*, *Lomentaria*, *Gastroclonium*, *Chylocladia*.

[d] Group 4 (Calcareous encrusters): *Lithophyllum*, *Mesophyllum*, *Lithothamnion*.

Table 2 – Scores for the amount of opportunistics correction factor and modified RSL quality categories classification boundaries

Amount of green opportunistics[a]	Amount of red–brown opportunistics[a]	RSL-Q quality categories[b]
5 (0)	10 (0)	≥25 (High)
4 (1)	8–9 (1)	20–24 (Good)
3 (2)	6–7 (2)	14–19 (Moderate)
2 (3)	5 (3)	10–13 (Poor)
≤1 (4)	≤4 (4)	≤9 (Bad)

[a] The values are denoted as amount (score).
[b] The values are denoted as score (quality).

species, by estimating the amount of green, red and brown opportunists, using a five ranged semi-quantitative scale as suggested by Pedersen (personal communication to NEA GIG, 2006). In this work the following ranges have been considered: 0 absence, 1 very low, 2 low, 3 moderate, 4 elevated and 5 high abundance of opportunistics. The boundaries and the corresponding values for the amount of greens and red–browns (grouped) are indicated in Table 2, together with the modified boundaries for the final quality categories classification, after the addition of the scores obtained by the quantitative correction factors to the original RSL index. This is a preliminary approach that should be defined more precisely in order to reduce its subjectivity and to avoid problems associated to those cases where a total absence of vegetation or a great abundance of only one type of opportunistic species would produce high scores in these indicators.

In order to analyse the suitability of the CFR index and the RSL index in the ecological assessment of intertidal and shallow subtidal areas, correlation analyses were performed between the final scores obtained by each method and the expected results estimated a priori for each sampling station along the pollution gradients. These analyses were previously performed for the gross values, the calculated values and the single quality scores of each indicator, in order to assess significant correlations ($p < 0.05$) between all the scoring system elements and the expected results.

Additionally, to test the degree of agreement achieved by each of the two methodological approaches (CFR and RSL), weighted kappa analyses were performed between the obtained and the expected quality classifications considering five possible quality classes (bad, poor, moderate, good and high). Finally, the correlations between the final results of the CFR index and those obtained with the different alternatives of the RSL index were analysed, together with weighted kappa analyses, to see the degree of adjustment between both methodologies and to study the possibilities and requirements for the intercalibration of both methods. The criterion followed for the estimation of the degree of agreement was based on the scale proposed by Monserud and Leemans (1992), which is comprised of eight levels ranging from no agreement (kappa < 0.05) to a perfect agreement (kappa > 0.99).

3. Results

In general terms, both methods were sensitive to the different pollution gradients, obtaining worse quality values at those stations located closer to the pollution sources. In this sense, the CFR index had better correlation results than the RSL-external alternatives of the RSL index, but similar to those of the RSL-loc-Q alternative. Nevertheless, regarding the degree of agreement between the observed and the expected results, the RSL index showed a considerable overestimation of the quality values, producing a ‘‘poor–fair’’ prediction level and a low percentage of correctly classified stations. In contrast, the CFR index obtained an ‘‘excellent’’ prediction level and a high percentage of correctly classified stations. These results are analysed in detail in the following sections.

3.1. *CFR index*

The results for gross values of each indicator showed that pollution gradients were clearly associated to three of the four indicators; the general coverage of characteristic macroalgae (ranging from 3% to 90%), the richness of characteristic macroalgae populations (ranging from 1 to 13) and the amount of opportunistic species (ranging from 80% to 5%), in all cases from the most polluted stations to the control stations,

Table 3 – Results of the application of the CFR index. B: bad, P: poor, M: moderate, G: good, H: high

Single quality scores							
Station	Cover	Richness	Opport.	State	CFR score	Observed classification	Expected classification
Liñera 1	30	15	5	15	65	G	M–G
Liñera 2	40	15	5	15	75	G	G
Liñera 3	40	15	15	15	85	H	H
Usgo 1	0	7	5	15	27	P	B–P
Usgo 2	0	11	5	15	31	P	P–M
Usgo 3	40	15	30	15	100	H	H
Ontón 1.1	10[a]	3	0	0	13	B	B
Ontón 1.2	10	11	5	3	29	P	P
Ontón 1.3	40	15	20	11	86	H	M
Ontón 2	40	15	15	15	85	H	G
Ontón 3	40	15	30	15	100	H	H

[a] The obtained score follows the addition of +10 points due to substrate structure.

Table 4 – Correlation results of the indicators gross values and single quality scores against the expected quality values

Gross values		Single quality scores	
Indicator	R^2	Indicator	R^2
Cover	0.78***	Cover	0.72***
Richness	0.74***	Richness	0.74***
Opportunists	0.64**	Opportunists	0.57**
State	0.44*	State	0.44*
		Final CFR	0.87****

The correlation between the CFR final scores and the expected quality values are also included. Significance levels are marked as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

respectively. On the other hand, the physiological state indicator only showed a marked quality gradient against the chemical discharge of Ontón. These results are in accordance with the single quality scores obtained by each indicator and with the final scores obtained by the CFR index (Table 3).

The correlation analysis between the gross values of each indicator and the expected quality values (Table 4) confirmed that macroalgae cover population richness and proportion of opportunistic species are the most sensitive indicators ($R^2 = 0.78$***, $R^2 = 0.74$*** and $R^2 = 0.64$**, respectively). Similar results ($R^2 = 0.72$***, $R^2 = 0.74$*** and $R^2 = 0.57$**, respectively) were obtained for the single quality scores (Table 4). Finally, the high correlation obtained between the CFR values and the expected values assigned "a priori" ($R^2 = 0.87$****) indicated a good discriminating capacity of the CFR index in relative terms (Table 4). Furthermore, in absolute terms, 73% of the stations were correctly classified, obtaining a weighted kappa value of 0.86, which corresponds to an "excellent" prediction level according to Monserud and Leemans (1992).

As can be seen in Table 3, Ontón 1.1 was the worst valued station, classified with a "bad" quality, followed by Ontón 1.2, Usgo 1 and Usgo 2, classified as "poor". As they did not attain the score for "good" quality, they would fail to fulfil the requirements of the WFD for this biological quality element. On the other hand, Usgo 3, Ontón 3, Ontón 1.3, Liñera 3 and Ontón 2 were the best valued stations, with "high" quality values. Liñera was the location with the best global quality, with a "good" value even close to the source of pollution, while Usgo and Ontón showed marked quality gradients from the most polluted stations to the least polluted ones.

3.2. *RSL index*

Correlations between RSL index indicators (gross, calculated or single quality scores) and the expected quality values for each station (Table 5), indicated that species richness was one of the most sensitive ones, showing a clear gradient from unpolluted sites, with maximum richness values (37 species), to polluted sites, with minimum values (four species) ($R^2 = 0.86$**** by RSL-external and $R^2 = 0.78$*** by RSL-local). The single quality scores obtained by this indicator also reflected this trend, giving notably higher scores to the unpolluted stations ($R^2 = 0.8$*** by RSL-external and $R^2 = 0.66$** by RSL-local). In both cases (calculated richness and single quality score for richness), the more complete macroalgae species list used by the RSL-external alternative gave better results than the reduced species list used by the RSL-local alternative, especially due to the higher richness values obtained at the unpolluted stations.

The proportion of greens reflected also a marked decrease from polluted to unpolluted sites (Table 5: $R^2 = 0.7$** by RSL-external and $R^2 = 0.62$** by RSL-local). This tendency was not due to the reduction in the gross number of green species, as it can be seen in their low correlation values ($R^2 = 0.33$ by RSL-external and $R^2 = 0.25$ by RSL-local), but to their relative amount in relation to total richness, including brown and red species. In contrast, the single quality score obtained by the proportion of red species was the least sensitive indicator, obtaining maximum values in all the analysed alternatives (R^2 = not available in both alternatives). In this case, there was a clear gradient in the number of reds between the good and bad stations ($R^2 = 0.92$**** by RSL-external and $R^2 = 0.85$**** by RSL-local), but their proportion varied between 40% and 76.5% of the total, and not always following a clear tendency between polluted and unpolluted stations. This rendered low correlation values with the expected qualities ($R^2 = 0.21$ by RSL-external and $R^2 = 0.12$ by RSL-local).

As with richness and number of reds, the number of species of ESG 1 increased from polluted to unpolluted sites ($R^2 = 0.69$** by RSL-external and $R^2 = 0.77$*** by RSL-local). However, the number of species of ESG 2 increased as well ($R^2 = 0.82$**** by RSL-external and $R^2 = 0.53$* by RSL-local), so the ESG ratio did not show a clear variation along the pollution gradient ($R^2 = 0.02$ by RSL-external and $R^2 = 0.22$ by RSL-local). The different ecological state groups assigned to the species by RSL-external in relation with RSL-local produced great differences in the final results of

Table 5 – Correlation results of the indicators gross values, calculated values and single quality scores against the expected quality values, for RSL-external and RSL-local evaluation alternatives

Gross values			Calculated values			Single quality scores		
Indicator	RSL-ext.	RSL-loc.	Indicator	RSL-ext.	RSL-loc.	Indicator	RSL-ext.	RSL-loc.
			Species richness	0.86****	0.78***	Species richness	0.80***	0.66**
Number of green species	0.33	0.25	Proportion of greens	0.70**	0.62**	Proportion of greens	0.66**	0.71**
Number of red species	0.92****	0.85****	Proportion of reds	0.21	0.13	Proportion of reds	N/A	N/A
Number of opportunists	0.28	0.19	Proportion of opportunists	0.55**	0.58**	Proportion of opportunists	0.15	0.20
ESG 1	0.69**	0.77***	ESG ratio	0.02	0.22	ESG ratio	0.01	N/A
ESG 2	0.82****	0.53*						

Significance levels are marked as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 6 – Estimated classifications for each station and final RSL index results obtained by each evaluation alternative

Station	Estimated classif.	RSL-external				RSL-local			
		Qualitative		Quantitative		Qualitative		Quantitative	
		RSL score	Observed classif.	RSL score	Observed classif.	RSL score	Observed classif.	RSL score	Observed classif.
Liñera 1	M–G	19	H	24	G	21	H	26	H
Liñera 2	G	18	G	23	G	20	H	25	H
Liñera 3	H	21	H	28	H	22	H	29	H
Usgo 1	B–P	13	M	18	M	16	G	21	G
Usgo 2	P–M	15	G	20	G	18	G	23	G
Usgo 3	H	16	G	24	G	18	G	26	H
Ontón 1.1.	B	9	M	14	M	13	M	18	M
Ontón 1.2.	P	17	G	24	G	17	G	24	G
Ontón 1.3.	M	20	H	28	H	17	G	25	H
Ontón 2	G	20	H	25	H	22	H	27	H
Ontón 3	H	16	G	24	G	19	H	27	H

B: bad, P: poor, M: moderate, G: good, H: high.

the different evaluation alternatives, thus higher values were obtained with the latter, because many of the species classified as ESG 2 by RSL-external (e.g. *Gelidium* sp., *Stypocaulon* sp., *Gigartina* sp.) were classified as ESG 1 by RSL-local. As a result, in the case of RSL-local, all stations got the highest scores for the ESG ratio indicator, because the number of species classified as ESG 1 was generally higher than the number of species of ESG 2. In any case, the obtained scores for the ESG ratios did not correlate well with the expected quality values in any case ($R^2 = 0.01$ by RSL-external and R^2 = N/A by RSL-local).

Regarding the proportion of opportunistic species, the results were very similar to those obtained by the proportion of greens ($R^2 = 0.55^{**}$ by RSL-external and $R^2 = 0.58^{**}$ by RSL-local), showing that most of the identified opportunistic species were green species. Additionally, the low range of scores established for this indicator (only three ranks), produced a bad correlation between the obtained single quality scores and the quality values of the stations ($R^2 = 0.15$ by RSL-external and $R^2 = 0.2$ by RSL-local).

The shore description parameter is not a biological indicator, but it acts as a correction factor to compensate for the different settling suitability of the species. The results show a small variation associated to this parameter, giving values of one or two in all cases.

Finally, the inclusion of quantitative aspects, although showing bad correlations with the expected quality values at individual indicator level (ranging from $R^2 = 0.02$ to $R^2 = 0.24$), produced a notable improvement of the final results (Table 6) by relatively reducing the RSL scores values of some stations located close to the pollution sources (e.g. Liñera 1) and increasing the scores in others located further away from the pollution sources (e.g. Usgo 3). The only exception was the case of Ontón 1.3, where the inclusion of the quantitative aspects led to a worsening of the final results.

When comparing the different pollution types or locations, Liñera had the best global quality, with a ''good'' value even at its most polluted stations (Table 6), while Usgo and Ontón showed a marked gradient of quality from the most polluted stations, in most cases classified with ''moderate'' quality values, to the healthiest ones, with ''high'' quality values. Liñera 1 and Ontón 1.3 showed exceptions from the common pattern obtaining markedly higher values than what was expected. On the other hand, Usgo 3 and Ontón 3 had, in some cases, lower scores than expected.

In accordance to the results obtained by the CFR index, Ontón 1.1 and Usgo 1 were in all cases the stations with the lowest quality scores (Table 6), classified as ''moderate-good'', and followed by Ontón 1.2 and Usgo 2. According to these results, only Ontón 1.1 and Usgo 1 obtained quality values below the ''good'' category, by most of the four alternatives, and therefore they would fail to fulfil the minimum quality required for this biological element by the WFD. These stations are located nearest to the industrial pollution sources. On the other hand, Liñera 3 and Ontón 2 were the best valuated stations, obtaining ''high'' qualities by all the applied alternatives.

In the case of quantitative alternatives, Liñera 3 was still the highest scoring station. Usgo 3, Ontón 1.3 and Ontón 3 were the stations with the highest improvements of their quality values due to the incorporation of the quantitative aspect. These stations represent the expected highest quality points at each location.

In relative terms, the correlations between the observed and the expected quality scores were low, though the inclusion of quantitative indicators produced a significant improvement of the correlations (Table 7). This improvement was higher in the RSL-local alternatives ($\Delta R^2 = 0.23$) than in the RSL-external alternatives ($\Delta R^2 = 0.09$). Among all, the RSL-loc-Q alternative obtained the best results, reaching an $R^2 = 0.81^{***}$.

Furthermore, in absolute terms, considering the exact classifications assigned to the stations, a significant over-

Table 7 – Correlation results between the expected and the obtained quality values, weighted kappa values and corresponding prediction levels for each evaluation alternative

	RSL-ext.	RSL-loc.
Qualitative	0.42*/0.404 (fair)	0.58**/0.444 (fair)
Quantitative	0.51*/0.395 (poor)	0.81***/0.453 (fair)

Significance levels are marked as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

estimation was detected in stations located closest to the pollution sources. This overestimation produced an incorrect quality assignation in most of the analysed stations, giving correct classification percentages between 18% (by qualitative alternatives) and 27% (by quantitative alternatives). The results of the weighted kappa analysis for each alternative are shown in Table 7. As represented, the RSL-local alternatives produced closer results to the estimated ones, but the weighted kappa values were still very low, corresponding to a ''fair'' prediction level according to Monserud and Leemans (1992).

3.3. *Intercomparison between the CFR index and the RSL index*

As observed in Fig. 2, the results obtained by the CFR index and the RSL-external and RSL-local qualitative alternatives were poorly correlated ($R^2 = 0.47^*$ and $R^2 = 0.43^*$, respectively). However, the correlations between the CFR and the RSL quantitative alternatives were notably higher, especially in the case of RSL-local-Q alternative ($R^2 = 0.72^{**}$).

In absolute terms, the results of the weighted kappa analysis involving both methodologies were still very low, with percentages of agreement ranging between 27% and 45% and ''fair'' prediction levels in all alternatives (Table 8).

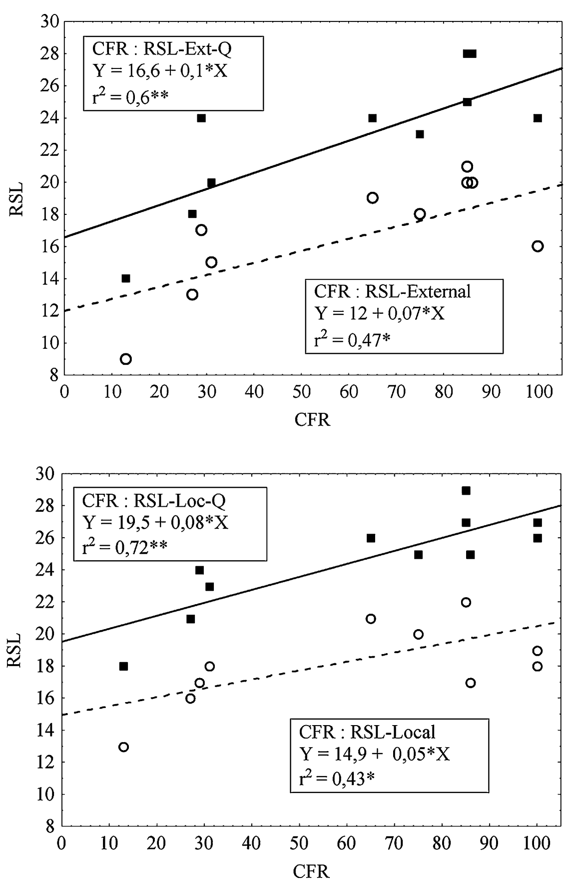

Fig. 2 – Correlations between the results obtained by the CFR index and the different alternatives of the RSL index. Significance levels are marked as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 8 – Weighted kappa values and corresponding prediction level between the results obtained by the CFR index and the different alternatives of the RSL index

	RSL-ext.	RSL-loc.
Qualitative	0.522 (Fair)	0.427 (Fair)
Quantitative	0.527 (Fair)	0.531 (Fair)

4. Discussion

As it has been seen, the CFR index responded rather accurately to the analysed pollution gradients. Additionally, the different alternatives of the RSL, modifying the ESGs (RSL-local) and including quantitative aspects (RSL-loc-Q), produced a significant improvement of the results, which constitutes a preliminary evidence as to the intercalibration possibilities of both indices in the geographical context of the north coast of Spain. The general overestimation of the ecological quality of the stations is the main aspect that should be tackled for a better adjustment of the RSL index, especially in those stations corresponding to most polluted sites.

At this point, different questions arise regarding possible explanations for the divergence of the results from the expected ones: are the selected indicators appropriate for accomplishing the requirements of the water framework directive? Are they sensitive enough for detecting changes in the ecological quality along the different environmental gradients? Do global indices integrate the environmental implication and importance of each indicator correctly?

Regarding the first question, requirements of the WFD for this biological quality element (rocky shore macroalgae) include the assessment of all disturbance-sensitive macroalgal taxa with consistent levels of cover and abundance respect to undisturbed conditions. In this sense, it seems clear that three of the four indicators included in the CFR i.e. cover, richness and presence of opportunistic or pollution indicator species, fulfil the requirements of the WFD statements and have proved to match adequately the different pollution gradients. Meanwhile, the RSL index, which only considers richness of macroalgae and different derived indicators according to proportions of taxonomic (reds–greens), ecological (ESGs) or pollution-tolerant (opportunistic) groups, is not fully in accordance with the WFD requirements.

Several authors have related the amount of macroalgae and the proportion of late-successional versus opportunistic species as an indicator of the ecological status of ecosystems (Orfanidis et al., 2001; Krause-Jensen et al., 2007), suggesting that the higher abundance of perennial or pollution sensitive species and the lower proportion of opportunistic or pollution indicator species are indicative of good ecological quality. Therefore the quantitative assessment of the cover percentage of characteristic macroalgae populations, basically sensitive and perennial species, could be considered as an appropriate indicator for the assessment of the ecological quality, since there were very significant correlations between these and the pollution gradients in the current study ($R^2 = 0.78^{***}$).

Alternatively, the proposal of Pedersen (personal communication to NEA GIG, 2006) to include into the RSL index a quantitative correction factor for the amount of opportunistic species, has notably increased the correlations between the observed and the expected results and, to a lesser extent, the percentage of correctly assigned categories to the stations. However, to achieve a higher improvement in the assignation of categories, a slight modification of the scores assigned to this indicator would be advisable for this coastal zone.

Regardless of the requirements of the WFD, a more important question to address is the sensitivity of the indicators, included in the CFR and the RSL proposals, to detect pollution gradients. The results of the current study demonstrate, firstly, that not all the variables showed a clear relationship with pollution gradients and, secondly, that transformation procedures (ranks, scoring system) may play an important role in the significance levels of gross values, calculated values and single quality scores, and seem to be a critical factor for adjustment of global ecological status scores at each station (Table 5).

In relation to pollution sources, the species richness indicator produced the most homogeneous and accurate results for both indices, even for the different alternatives of the RSL index (external and local). Considering only the latter case, the quality scores were similar among alternatives, differing only by one quality level in 3 of the 11 stations. Although richness is not accounted for in relative values, the use of different number of species at each RSL alternative (RSL-external: 81 spp.–RSL-local: 50 spp.) did not produce a significant difference in the quality scores of this indicator. In addition, the proportions of green and red species were very similar for both alternatives, indicating that the reduced species list proposed for the RSL-local alternative is in agreement with the complete list (RSL-external).

Beyond the between-indices intercalibration aim, a more interesting aspect from an ecological point of view is the different approach used for the estimation of richness between the CFR and RSL indices. While the latter one is based on the assessment of the total specific richness, including both sensitive and tolerant species (''taxonomic approach''), the CFR index only estimates richness scores from the conspicuous presence of characteristic macroalgae populations (>1% cover), that make up the zonation pattern (''ecological approach''). This latter approach is in agreement with the suggestions of Eriksson and Bergström (2005), to use perennial species for monitoring purposes. Anyway, the results from both types of approaches gave similar quality scores, showing that significant contamination effects may be reflected at the both scales, the species and the ecosystem level.

Regarding the different pollution gradients, population richness showed lower variations than specific richness at urban discharges (Liñera), but final quality scores for both indicators gave the highest values, which may indicate that this type of disturbance neither reduces the number of species nor the characteristic populations. Conversely, richness differences between stations along severe industrial point sources showed a clear gradient, producing good global correlation values (Tables 4 and 5).

Consequently, the good correlations of this indicator between the observed and the expected results ratify its suitability and that of the established scoring system proposed in both indices for pollution assessment. On the other hand, sampling effort and expertise needed for assessment of specific richness is much greater than those needed for the evaluation of characteristic population richness.

A less homogeneous pattern was observed when the proportion of opportunistic species was assessed by the two indices along pollution gradients. There are several plausible explanations for this difference. Like with richness, the different approaches used for the estimation of opportunistic species by both indices is the main cause. While the RSL index evaluates the degree of opportunistic species from a taxonomic point of view (specific proportion of opportunists), the CFR index uses an ecological approach that considers the amount of opportunistic species in terms of relative cover of these species respect to the total vegetated surface. From our results, it can be concluded that the inclusion of a quantitative evaluation of the opportunistic species coverage used in the ecological approach produced better results than the taxonomic approach, especially when the single quality scores are considered. In a similar way, the proposal of Pedersen (personal communication to NEA GIG, 2006) to include a semi-quantitative evaluation of opportunistic species, notably improved the results of the RSL index.

The proportion of green species and the ESG ratio indicators can be considered similar to the proportion of opportunist species and therefore one should determine if these indicators are redundant or not. As stated by Wells et al. (2007), they incorporate different aspects of community composition hence they should respond differently to various environmental stresses. However, the results obtained at the single quality scores level showed that only the proportion of greens resulted in good correlations with the pollution gradients. In the case of the proportion of opportunists, significant correlations were observed at the calculated values level but not when the scoring system was applied. The ESG ratio did not correlate well even at the calculated values level, because both the number of ESG I and ESG II species increased towards the unpolluted stations, reducing the reliability of the ESG ratio. This effect was especially noticeable in the RSL-external alternative, indicating that some species could have been misclassified into the two ESGs, as was also suggested by Wells et al. (2007). The improvements in the results obtained with the ESGs assigned in the RSL-local alternative strengthen this idea, but they are still insufficient to justify the usefulness of the ESG ratio indicator in order to assess pollution effects.

If we analyse the reasons that may have caused the deviations from the expected results, one must consider that the assigning of ESGs was based on the concept of morphological and functional-form groups, as described by Littler and Littler (1980, 1984) and later adapted by Orfanidis et al. (2001) to divide the species into two groups. But this hypothesis was not specifically proposed to predict resistance to pollution (Arévalo et al., 2007) and consequently many of the species classified as ESG II in the RSL-external alternative, generally corresponding to the coarsely branched functional-form group, could have been classified as ESG I due to their mid-late successional character and their longevity. When those species were considered as ESG I in the RSL-local alternative, a higher overestimation of the RSL score was observed due to the increase of the ESG ratio; however, the correlation with the expected quality values was improved, which suggests that they could be more appropriate, at least in this coastal region.

Anyway, considering that most of the identified opportunistic species were green species and that the ESG ratio indicator does not reflect clearly the effects of pollution, it seems recommendable to reduce the apparent redundancy associated to these three indicators by selecting a unique one that adequately reflects the pollution effects. In this sense the proportion of opportunistic or green species or an alternative indicator based on a sensitive/tolerant species ratio, might be more appropriate provided that an adequate scoring system is used. In that case, it would be necessary to accurately identify which species belong to each of these sensitivity groups.

The physiological state indicator of the CFR index showed significant correlations with the expected values, however, the high scores obtained at Liñera and Usgo lessen the usefulness of this indicator. On the other hand, the good results obtained at Ontón could be a justification for the maintenance of this indicator. Maybe the most important problem associated with this indicator is the difficulty to objectively discriminate between natural and anthropogenic causes of the effects on the macroalgal communities and their evaluation.

The shore description indicator (geomorphological and physical aspects of habitats) of the RSL and CFR indices acts as a correction factor for the settlement of macroalgae and seems to be prerequisite for correctly evaluating macroalgae assemblages. In contrast, the presence of invasive species, considered in the CFR index as a penalizing factor, is not necessarily related to pollution and therefore its use for the assessment of macroalgae communities in relation to water quality is questionable. This is at least the case of *Sargassum muticum*, whose extensive presence and difficult eradication (Critchley et al., 1986) makes it more of a characteristic species rather than an invasive one.

The last question to be answered refers to the suitability of global indices to detect changes along the pollution gradients. It seems that the type and the number of indicators included in the CFR index are good enough to reflect the ecological status of macroalgal communities. Furthermore, the final scores showed a good correlation with the expected qualities in the three areas under study. On the other hand, the redundancy of some indicators (greens, opportunistics, ESG ratios) in the RSL index might result in an overrepresentation of one of the environmental features, which anyway did not correlate consistently with the expected quality gradients.

The intercomparison analyses carried out between the different alternatives of the RSL index and the CFR index showed significant correlations in all cases, but especially in the case of RSL-loc-Q. However, the agreement in quality categories assigned to the stations was very low in all cases. One of the reasons for these results could have been due to the high qualities obtained by the RSL alternatives, especially in the most polluted stations.

The observed misadjustments between the expected and the obtained results of the RSL index could be associated to the differences existing in the intertidal algae community composition between northern cold waters, where brown algae are dominant, and southern temperate waters, where red algae predominate (Fischer-Piette, 1963; Lüning, 1990; Boaventura et al., 2002). Although the water framework directive considers the Northeast Atlantic as an entire ecoregion, the large marine ecosystems project (LME), initiated to support the global objectives of Agenda 21, clearly distinguishes the Iberian coastal marine ecosystem from northern coastal areas (EEA, 2006) and therefore could justify the classification of different coastal types and consequently the adoption of different assessment approaches.

The better results obtained in Liñera comparing to Usgo and Ontón, could have been due to the less harmful nature of the urban discharges comparing to the industrial effluents. Thus, the effects of the latter ones produced a notable reduction in the richness and cover of macroalgae, especially of sensitive species, while the effects of the urban discharges were more associated with the proliferation of green and opportunistic species, without an apparent reduction in cover or richness. The good values found for physiological state and species richness in Usgo, indicate somehow that the intertidal macroalgae communities are recovering in the stations located close to the old discharge point. However, the likely effect of the calcium chloride sludge on features such as turbidity or light penetration of the whole water body, may explain the low cover values and consequently the overall ‘‘poor–moderate’’ quality value of these stations. In the case of Ontón, the marked quality gradient indicates that the communities are highly affected close to the discharge point but the recovery increases rapidly with increasing distance from it. The high hydrodynamism of the Cantabrian sea (Valencia et al., 2004; Castanedo et al., 2006) and the exposed character of the coastal zone around Ontón, are likely to contribute to a great extent to the fast dilution of the fluoride concentration and the recovery of macroalgae communities not far from the discharge point.

Finally, for both RSL and CFR indices, the evaluation of the ecological status of macroalgae communities is based on the analysis of visual surveys of richness, cover, presence of opportunistic species or apparent physiological status. However, there could be other kind of damaging effects not directly detected by visual assessments, like reduced growth rates, reproductive abnormalities or other physiological alterations (Eklund and Kautsky, 2003). In this sense, the high variability of industrial effluents discharging to the coastal zone, highlight the need for developing environmental risk analyses and the design of case specific operational controls.

5. Conclusions

As a conclusion, it can be said that the CFR index responded more accurately than the RSL index to the pollution gradients analysed. Furthermore, the easy application and the reduced processing time of the CFR index provide an additional argument for its use. On the other hand, the CFR index is more subjective than the RSL index and is not as precise due to the ranks based scoring system.

To achieve good calibration and validation of both indices, further analyses and intercalibration should be carried out at different geographical locations and against different types of pollution sources. In all cases, the preliminary results obtained in this study show that the intercalibration of both indices is possible after certain adjustments or modification of some of the above mentioned aspects.

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