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Testing taxonomic resolution, data transformation and selection of species for monitoring macroalgae communities

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Abstract

The Water Framework and Habitats Directives require the evaluation of both the conservation and ecological status of macroalgae communities at water body or habitat level. However, assessments of macroalgal communities are highly time-consuming, both in terms of sampling effort and laboratory processing. These constraints have brought about their oversight in many marine monitoring programs, especially in subtidal environments. By using data from intertidal and subtidal macroalgae assemblages of Mouro Island (North coast of Spain) we wanted to identify possible cost-effective methods for monitoring this biological indicator, based on both high taxa levels and use of representative taxa. Multivariate analyses were applied using different data transformations. The results show that macroalgal communities are robust to aggregation to genus or even family level. Moreover, the outcomes show that transformation types introduce higher variation in the multivariate pattern of samples than the taxonomic level to which organisms are identified. Also, the study supports the use of representative taxa as a surrogate to overall community structure. Therefore, we conclude that a rapid-assessment by means of field evaluations, based on coverage of representative taxa, is a reliable alternative for the assessment of macroalgae status. In addition this procedure allows evaluation at a broader spatial scale (water body or habitat level) than traditional quantitative sampling procedure does.

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1. Introduction

Increasing concern regarding conservation of aquatic systems, including marine environments, has culminated in the promulgation of two important European Directives: the 2000/60/EC Water Framework Directive (EC, 2000), specifically focused on the protection and management of water bodies, and the 92/43/EEC Habitats Directive (EEC, 1992), related to the conservation of natural habitats and of wild fauna and flora. To evaluate the accomplishment of their objectives, a systematic assessment of biological communities is required. In this context, benthic communities, including

macroalgae, are obligatory biological elements when monitoring European coastal waters and evaluating their ecological and conservation status.

However, the difficulties inherent to the assessment of benthic communities in coastal waters are well known, due to their natural variability, their high sampling and laboratory processing effort and the insufficient knowledge about the structural and functional characteristics of that ecosystem (Warwick, 1993). These problems increase in the case of rocky substrates and mainly in subtidal bottoms, because of the limitations of diving works for direct sampling (depth, time, weather conditions). Consequently, considerable attention has been paid to the establishment of suitable and cost-effective methods for monitoring benthic communities (Warwick, 1988a,b).

Numerous papers have dealt with the "taxonomic sufficiency" required for the detection of changes in benthic

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communities (Ellis, 1985) and their validity in impact assessment of marine environments (Warwick, 1988a,b; Ferraro and Cole, 1990; Gray et al., 1990; Ferraro and Cole, 1992, 1995; Somerfield and Clarke, 1995; Karakassis and Hatziyanni, 2000). This approach is based not only on the reduction of effort, but also on the increase of our ability for detection of changes and the precision of data acquired (Warwick, 1993). In this sense, the Water Framework Directive specifies the following: "In selecting parameters for biological quality elements Member States shall identify the appropriate taxonomic level required to achieve adequate confidence and precision in the classification of the quality elements". Therefore, the concept of taxonomic sufficiency should be considered in the design of monitoring programs of coastal waters, even though nowadays there is no agreement regarding the validity of such an approach.

Much less attention has been paid to statistical analyses, in spite of their importance for final results. Furthermore, in many cases data treatment depends more on scientist's experience or available software, rather than on an ecological basis. Multivariate statistical analyses have been frequently applied because of their reliability and robustness regarding benthic marine communities (Clarke and Warwick, 2001). One relevant aspect of multivariate analysis is the transformation of data. Decision on which transformation is the most appropriate should be based both on the nature of the data and the specific objective of the study in question. However, regardless of the controversy related to taxonomic resolution, data transformation in statistical analysis of benthic communities has aroused little interest, despite the fact that its effects can be as important in determining the outcome of multivariate analysis as the taxonomic level to which organisms are identified (Clarke and Green, 1988; Olsgard et al., 1997, 1998; Legendre and Gallagher, 2001; Anderson et al., 2005).

Most papers that consider taxonomic resolution refer to soft bottom fauna, while this concept is rarely applied to rocky bottoms (Smith and Simpson, 1993; Juanes and Canteras, 1995; Urkiaga-Alberdi et al., 1999; Pagola-Carte et al., 2002; Anderson et al., 2005). Macroalgae are the main structural element of "coastal reefs", protected habitats within the Habitats Directive (type reference: 1170), and one of the key biological elements required for monitoring coastal waters according to the WFD. Hence, more research is necessary to test if the general conclusions obtained from research on soft bottom macroinvertebrates are also suitable for macroalgae assemblages. In addition, it is necessary to explore the utility of using lower taxonomic resolution to reflect species level diversity in unperturbed areas for conservation purposes (Vanderklift et al., 1998).

The need of using quantitative (abundance or biomass), qualitative (presence-absence) or semi-quantitative measures (e.g. frequency, relative coverage, etc.) has been a matter of debate. The quantitative approach has been extensively used and provides relatively detailed information concerning composition and structure of benthic communities. However, it is labour-intensive and has often sampling constraints, which is likely to be a major limitation in subtidal rocky communities. The qualitative or semi-quantitative assessments are less precise but much more cost-effective. Their major advantage is a rapid field assessment without sampling. This option is especially feasible if macroalgae are the target organisms. Macroalgae assemblages are characterized by the dominance of a few species, in most cases easy to identify "in situ", and the presence of many others, more difficult to identify to species level, but with a relatively low percentage of the total biomass. Moreover, this approach provides a broader and more representative image of the ecosystem itself, given that the assessment is not restricted to point samples as quantitative methods require. In this respect, it is noteworthy that both the WFD and the Habitats Directive require broad water body and habitat evaluations. Thus, the establishment of tools that provide an overall assessment of conservation and ecological status is required. According to the WFD the use of qualitative or semi-quantitative indices focusing on previously selected representative species has been proposed for the assessment of the ecological status of macroalgae (Ballesteros et al., 2007; Wells et al., 2007; Juanes et al., in press).

On the other hand, we have to bear in mind that the main purpose of a marine monitoring program is to detect harmful effects in the ecosystem and to identify the pressure that causes the impact. We should not mistake these objectives with those of pure scientific research, which usually requires more rigorous assessments. Consequently, for the monitoring purposes we just need to find out methodologies that allow the detection of impacts and the quantification of changes in marine communities (positive or negative). Thus, the optimal evaluation system would consist of an approach fulfilling these objectives in the most feasible and cost-effective way. Of course, such monitoring programs should be done in relation to more exhaustive studies, hence providing both fundamental knowledge of the water environment and a long term assessment of changes in natural conditions (referred to as "surveillance monitoring" in the WFD).

Taking into account the aforementioned aspects, this paper aims: (1) to test the suitability of using reduced subsets of representative species for the assessment of conservation or ecological status of macroalgae assemblages; (2) to analyze the robustness of taxonomic aggregation; (3) to evaluate the effects of different types of previous transformations of data in subsequent statistical analyses; and (4) to identify possible cost-effective methods for monitoring macroalgae, based on the aggregation of species and the use of reduced subsets of taxa.

2. Materials and methods

The data used were collected during an extensive project on the biodiversity of benthic communities of Mouro Island (García-Castrillo et al., 2000a,b; Puente, 2000; Arroyo et al., 2004; Preciado and Maldonado, 2005; Serrano et al., 2006). The study site is a well-preserved area located on the north coast of Spain, in the mouth of the Bay of Santander, one of the largest estuaries in the Cantabrian sea (Fig. 1). Rocky reefs dominate the bottom of the island, ranging from median to high wave exposure and maximum depths of 20 m. Two



Fig. 1. Study site.

macroalgae assemblages were selected from the variety of communities analyzed in that area. One data set corresponds to a subtidal assemblage dominated by the brown seaweed *Laminaria ochroleuca* (28 samples), a common species in the shallow waters of the Cantabric sea. The other data set is based on 18 samples taken at the mid-intertidal level, where the structural species is *Corallina elongata*. These assemblages constitute one of the most representatives of the subtidal and intertidal environments, respectively, on North East Atlantic European shores (Fernández and Niell, 1982), and thus might be good indicators of the general status of macroalgae in these coastal areas.

The samples were obtained by direct sampling, taking replicates of 2500 cm^2 ($50 \times 50 \text{ cm}$) in the subtidal (*Laminaria* assemblage) and 625 cm^2 ($25 \times 25 \text{ cm}$) in the intertidal (*Corallina* assemblage) (Hiscock, 1979). In each replicate, macroalgae were identified to species level and the biomass quantified (g dw m⁻²) (Brinkhuis, 1985). The nomenclature follows the check-list included in the European Register of Marine Species (Guiry, 2001).

The treatment of data comprised the application of different factors of variation to the raw biomass data sets of both communities. The first factor analyzed was the suitability of using subsets of representative species for the assessment of macroalgae assemblages. Therefore, new reduced matrices were created for each community by removing species with low biomass (minimum weight of 0.5 g dw m^{-2}) (Fig. 2). With this treatment we tried to simulate a rapid field assessment, where only the most conspicuous species can be identified "in situ". The second factor considered was the effect of using different taxonomic levels in assessing the macroalgae assemblages. Hence, both species matrices (full data set and subset of more abundant species) were aggregated to genus, family and order levels. The third factor tested was the effect of transformations in the multivariate analyses. Thus, each of the matrices obtained previously were square root, fourth root, log(x + 1) and presence/absence transformed, including not transformed data in the following analyses.

Consequently, we obtained 40 matrices for each assemblage (full data sets or subsets, 4 taxonomic levels, 5 types

of data transformation). The similarities among samples were calculated for each matrix by means of the Bray–Curtis similarity index (Bray and Curtis, 1957). Resemblances among all matrices were determined by the Spearman rank correlation coefficient (Clarke and Warwick, 2001). Further comparisons among multivariate patterns were visualized by means of a second-stage non-metric multi-dimensional scaling ordination (Somerfield and Clarke, 1995). This method is appropriate to examine the degree of resemblance among similarity matrices obtained with different multivariate approaches.

Also, we explored the effect of the various treatments in the detection of environmental gradients. According to previous studies, the main environmental gradients operating in the study site are determined by wave action in the intertidal and by depth in the subtidal (García-Castrillo et al., 2000a; Puente, 2000). Thus, for the intertidal samples ("*Corallina*" assemblage) we looked at differences among sites i.e. exposed and semi-exposed, while for the subtidal ("*Laminaria*" assemblage) set of samples, we examined differences among samples above and below -10 m. Non-metric Multidimensional Scaling analyses (nMDS) (Kruskal and Wish, 1978) were performed on various similarity matrices from both assemblages. Formal significant test for differences between groups of samples were tested by means of Analysis of Similarities (ANOSIM) (Clarke, 1993).

The savings in processing effort derived from aggregating species into higher taxonomic levels or by the use of subsets of representative taxa were estimated by following the proposal of Ferraro and Cole (1995). They proposed that the reduction in time as a consequence of aggregation in higher taxonomic levels (or selection of representative species in our case) could be estimated by taking the ratio of the number of higher taxa to the number of species, assuming that the time to identify the taxa is directly related to the number of categories in which they must be placed. Also, the balance between the loss of information and the reduction in taxonomic effort was calculated by means of the cost/benefit index proposed by Karakassis and Hatziyanni (2000), the formulation of which is as follows:



Fig. 2. Methodological procedure followed for the data treatment.

$$CB_{L} = \frac{(1 - r_{L})}{\frac{S - t_{L}}{S}}$$

 CB_L is the cost/benefit ratio at taxonomic level L, r_L is the Spearman correlation coefficient between taxonomic level L and species level, t_L the number of taxa present at taxonomic level L and S the number of species. Therefore, the lower the index value, the higher the cost-effectiveness of the treatment analyzed is. The CB_L ratio was calculated for each type of data transformation.

Statistical analyses were carried out using the PRIMER-E statistical package (Clarke and Warwick, 2001).

3. Results

3.1. General description of the communities analyzed

In the "*Corallina*" assemblage 63 species were identified, grouped into 40 genera, 18 families and 14 orders. The number of species (67) and orders (18) registered in "*Laminaria*" were similar, although the numbers of genera (57) and families (30) were higher than in the intertidal assemblage. In both communities, most of the identified species belonged to the phylum Rhodophyta (75% and 85% of the species in "*Corallina*" and "*Laminaria*", respectively).

The number of taxa diminished noteworthy by considering only those with a biomass higher that 0.5 g dw m^{-2} in some

sample, especially regarding species and genera. Thus, in the "*Corallina*" assemblage the reduce matrices comprise 10 species, 10 genera, 9 families and 8 orders, and in the subtidal one ("*Laminaria*") the selected taxa are distributed in 30 species, 27 genera, 19 families and 13 orders.

The biomass values were highly variable in both communities, with mean values of 248 ± 208 g dw m⁻² in "Corallina" and 538 ± 240 g dw m⁻² in "Laminaria". The dominance of the structural species was noticeable, followed by a low biomass of the remaining taxa (mean weights of 33 ± 43 and 48 ± 82 g dw m⁻², if we exclude *Corallina elongata* or Laminaria ochroleuca from the biomasses estimation, respectively). Few species had a mean value higher than 1 g dw m^{-2} , and only Caulacanthus ustulatus in the intertidal and Sacchoriza polyschides in the subtidal exceeded a mean weight of 10 g dw m⁻² (Table 1). Therefore, only 10 species in "Corallina" and 30 in "Laminaria" showed biomasses up to 0.5 g dw m^{-2} in some of the samples. According to the criteria established (see Section 2), these species constitute the subsets of representative species used in the subsequent analysis (reduced matrices).

Also it is important to analyze the number of taxonomic units in each taxa, i.e. number of families, genera and species included in each order, family and genus, respectively. As a synthesis of the taxa distribution model, Table 2 shows the number of taxa that include 1, 2 or more than 2 lower taxonomic levels. As can be seen, most taxa include only 1 Table 1

| Assemblage | Phylum | Order | Family | Species | $\text{Mean} \pm \text{SD}$ | Maximum |
|-------------|-------------|------------------|-----------------|-------------------------------|-----------------------------|---------|
| "Corallina" | Rhodophyta | Corallinales | Corallinaceae | Corallina elongata | 215.71 ± 188.06 | 634.11 |
| | | Gigartinales | Caulacanthaceae | Caulacanthus ustulatus | 24.34 ± 38.42 | 119.81 |
| | | Ceramiales | Ceramiaceae | Ceramium ciliatum | 1.59 ± 4.49 | 16.02 |
| | | | | Pleonosporium borreri | 0.07 ± 0.22 | 1.00 |
| | | Gelidiales | Gelidiaceae | Gelidium latifolium | 0.36 ± 0.68 | 2.66 |
| | Phaeophyta | Sphacelariales | Stypocaulaceae | Halopteris scoparia | 1.34 ± 4.41 | 18.84 |
| | | | Sphacelariaceae | Sphacelaria cirrosa | 1.20 ± 4.53 | 19.82 |
| | | Ectocarpales | Ectocarpaceae | Ectocarpus sp. | 0.05 ± 0.22 | 1.00 |
| | Chlorophyta | Cladophorales | Cladophoraceae | Cladophora prolifera | 2.20 ± 5.28 | 19.31 |
| | | Codiales | Bryopsidaceae | Bryopsis plumosa | 1.20 ± 4.91 | 21.46 |
| "Laminaria" | Rhodophyta | Ceramiales | Rhodomelaceae | Pterosiphonia complanata | 6.05 ± 16.47 | 77.96 |
| | | | | Pterosiphonia ardreana | 0.023 ± 0.11 | 0.59 |
| | | | | Pterosiphonia pennata | 0.26 ± 1.30 | 7.03 |
| | | | | Pterosiphonia parasitica | 0.23 ± 0.73 | 2.93 |
| | | | | Boergeseniella thuyoides | 0.02 ± 0.13 | 0.69 |
| | | | Ceramiaceae | Halurus equisetifolius | 0.66 ± 1.70 | 6.23 |
| | | | Delesseriaceae | Cryptopleura ramosa | 0.46 ± 0.92 | 3.46 |
| | | | | Acrosorium venulosum | 0.41 ± 1.20 | 5.91 |
| | | | | Apoglossum ruscifolium | 0.06 ± 0.18 | 0.99 |
| | | | | Erythroglossum laciniatum | 0.03 ± 0.14 | 0.74 |
| | | | | Haraldiophyllum bonnemaisonii | 0.34 ± 1.28 | 6.78 |
| | | | Dasyaceae | Heterosiphonia plumosa | 0.04 ± 0.16 | 0.79 |
| | | Gelidiales | Gelidiaceae | Gelidium sesquipedale | 3.55 ± 9.35 | 41.86 |
| | | Rhodymeniales | Rhodymeniaceae | Rhodymenia pseudopalmata | 1.78 ± 2.57 | 11.17 |
| | | Plocamiales | Plocamiaceae | Plocamium cartilagineum | 1.16 ± 2.70 | 10.23 |
| | | Gigartinales | Cystocloniaceae | Calliblepharis ciliata | 0.69 ± 2.57 | 13.06 |
| | | 0 | • | Rhodophyllis divaricata | 0.08 ± 0.37 | 1.97 |
| | | | Phyllophoraceae | Phyllophora sicula | 0.023 ± 0.11 | 0.61 |
| | | Corallinales | Corallinaceae | Corallina officinalis | 0.37 ± 1.90 | 10.22 |
| | | Cryptonemiales | Kallymeniaceae | Callophyllis laciniata | 0.21 ± 0.43 | 1.48 |
| | | •• | · | Meridithia microphylla | 0.03 ± 0.13 | 0.73 |
| | | | | Kallymenia reniformis | 0.03 ± 0.11 | 0.58 |
| | | | Halymeniaceae | Cryptonemia seminervis | 0.15 ± 0.43 | 2.06 |
| | | Palmariales | Palmariaceae | Palmaria palmata | 0.07 ± 0.27 | 1.16 |
| | Phaeophyta | Laminariales | Laminariaceae | Laminaria ochroleuca | 489.43 ± 240.16 | 1292.27 |
| | 1 2 | | Phyllariaceae | Saccorhiza polyschides | 19.07 ± 61.82 | 312.35 |
| | | Fucales | Cystoseiraceae | Cystoseira baccata | 7.14 ± 30.20 | 159.11 |
| | | Dictyotales | Dictyotaceae | Dictyopteris membranacea | 5.14 ± 25.09 | 135.41 |
| | | Dictyosiphonales | Punctariaceae | Punctaria latifolia | 0.04 ± 0.19 | 1.03 |
| | Chlorophyta | Codiales | Codiaceae | Codium tomentosum | 0.03 ± 0.16 | 0.85 |

Mean (\pm SD) and maximum biomass values (g dw m⁻²) of the most abundant species in the "*Corallina*" and "*Laminaria*" assemblages (biomass higher than 0.5 g dw m⁻² in some sample). The families and orders which the species belong to are shown

taxonomic unit (family, genus or species), whereas those that comprise 3 or more are scarce. Those taxa that include more than 2 lower taxa are also shown.

3.2. Resemblance among matrices

The Spearman rank correlations obtained among the species similarity matrices and higher taxonomic levels using different transformations were significant and very high in most cases (p < 0.001), although some differences were found between the two communities analyzed (Fig. 3). In the "*Laminaria*" set of samples the correlations between the species and genus level matrices were almost perfect whichever transformation was applied. The correlations between species and family level matrices had a Spearman rank correlation

coefficient of r > 0.95 when applying none, square root and log(x + 1) transformation and a value of r = 0.88 was obtained by using the fourth root transformation. By using data sets at order level the multivariate pattern became quite different compared to lower taxonomic levels, although in the case of none and square root transformed data was relatively high (>0.85). In the case of "*Corallina*" samples, the correlations between species and higher taxa matrices resulted in a very high correlation coefficient when applying none, square root and log(x + 1) transformations, even at order level (minimum value of r = 0.98), but the *r* values decreased slightly with the fourth root transformation. In both communities, the use of qualitative data (presence/absence) produced major changes in the similarity matrices, except at the genus level in the case of "*Laminaria*".

Table 2

| Number of lower taxa | Orders | Families | | Genera | | |
|----------------------|------------|------------------|------------|---------------------|------------|-------------------|
| "Corallina" | | | | | | |
| 1 | 11 (78.6%) | | 10 (55.6%) | | 31 (77.5%) | |
| 2 | 2 (14.3%) | | 4 (22.2%) | | 5 (12.5%) | |
| 3 or more | 1 (7.1%) | Ceramiales (3) | 4 (22.2%) | Champiaceae (3) | 4 (10.0%) | Cladophora (4) |
| | | | | Corallinaceae (4) | | Callithamnion (5) |
| | | | | Rhodomelaceae (6) | | Polysiphonia (6) |
| | | | | Ceramiaceae (9) | | Ceramium (7) |
| "Laminaria" | | | | | | |
| 1 | 15 (83.3%) | | 22 (73.3%) | | 49 (86.0%) | |
| 2 | 1 (5.6%) | | 2 (6.7%) | | 6 (10.5%) | |
| 3 or more | 2 (11.1%) | Ceramiales (4) | 6 (20%) | Kallymeniaceae (3) | 2 (3.5%) | Callithamnion (3) |
| | | Gigartinales (8) | | Rhodomelaceae (3) | | Pterosiphonia (4) |
| | | | | Phyllophoraceae (4) | | |
| | | | | Corallinaceae (5) | | |
| | | | | Ceramiaceae (8) | | |
| | | | | Delesseriaceae (8) | | |

Number of orders, families and genera (percentage in brackets) that comprise 1, 2 or more than 2 lower taxa in the "*Corallina*" and "*Laminaria*" assemblages. Orders, families and genera that include more than 2 lower taxa are also shown (the number of taxa included in each one is shown in brackets)

On the other hand, the similarity matrices obtained with the full data set of species showed, in most cases, high correlations with those derived from the subsets of species, genus, families and orders (reduced matrices) (Fig. 3). The loss of information using these surrogates only became apparent in both assemblages if data were fourth root transformed (r < 0.85), being more acute the reduction in the correlation coefficient when using presence/absence data (r values around 0.3). The lower correlation obtained at order level in the subtidal community ("Laminaria") should also be pointed out.

The correlation patterns among the various treatments applied can be visualized in the second-stage MDS plot (Fig. 4). First of all, we can see a horizontal separation of groups for different transformations, from left (nontransformed data) to right (presence/absence data). Secondly, the symbols that represent matrices derived from species, genera, families and orders, using the same transformation, appear similar, although the distance among taxonomic levels increases as the transformation becomes stronger. Thus, with non-transformed data the matching among all treatments were absolute, while the dispersion using qualitative data (presence/absence) was noticeable. When using data based on orders, the main difference between "Corallina" and "Laminaria" assemblages resulted in a much greater loss of information in the latter. Also, it should be noticed that the dissimilarities among matrices obtained from the full data sets and from subsets of the most abundant species were only apparent when using fourth root and presence/absence data, while when applying the other transformations the differences were small.

3.3. Detection of environmental gradients

Figs. 5 and 6 include the MDS plots obtained from the full data sets and from the subsets of the most abundant species, using different transformations and taxonomic levels. To

illustrate the effects of species aggregation and the use of representative taxa, the ordination plot derived from the fourth root transformed data (all species) was chosen. As can be seen, the resemblance in the ordination patterns diminished with higher taxonomic levels. However, the overall pattern related to environmental conditions was retained both at genus and family level, and even when using order level in the case of the "Corallina" assemblage. The magnitude of the differences depends on the type of transformation applied. In other words, the type of transformation seems to produce higher divergences from the reference ordination patterns than when using species aggregation. Nonetheless, the plots derived from presence/absence data were more similar to fourth root transformed data than those obtained with raw or square root transformed data, despite the fact that the Spearman correlations showed a larger divergence than for the remaining treatments. On the other hand, the MDS plots of the "Laminaria" assemblage derived from the subsets of the most abundant taxa differ considerably from the full data set of species, despite Spearman correlations were high in most cases. On the contrary, the ordination of "Corallina" samples obtained from the representative species showed similar patterns in relation to wave exposure at all taxonomic levels.

The highest values of the ANOSIM statistic (R) in the "*Corallina*" assemblage were obtained with the full data sets at species level, using fourth root and presence/absence transformed data. Using these transformations, the R values diminished as the taxonomic level increased (Fig. 7). In general terms, the same results were obtained in the case of the "*Laminaria*" assemblage, but no differences were found between the values at species and genus levels (Fig. 8). Considering the remaining types of transformations in both communities (none, square and log(x + 1)), the ability of ANOSIM to detect differences among groups of samples was similar at all taxonomic levels, except at the order level in the subtidal



Fig. 3. Spearman rank correlations between full data set of species similarity matrices and those: (A) obtained at genus, family and order levels, applying different types of transformation; and (B) obtained from subsets of species, genera, families and orders (reduced matrices, biomass higher than 0.5 g dw m⁻² in some sample) (Sp = species, Gn = genus, Fm = family, Or = order, (r) = reduced matrices of taxa).

assemblage ("*Laminaria*"). It should be pointed out that all differences were significant, except in the case of non-transformed data.

The values of the R statistic, using the subsets of the most abundant species, were lower than those obtained with the full species and genus data sets using fourth root and presence/ absence data, but similar to those obtained with the remaining transformations or taxonomic levels (Figs. 7 and 8). Furthermore, in most cases ANOSIM discrimination among group of samples was similar at all taxonomic levels. Also, the differences derived from the type of transformation only became noticeable in the case of non- and square root transformed data in the subtidal set of samples.

3.4. Cost effectiveness

The estimations of savings in the processing effort, according to the method proposed by Ferraro and Cole (1995), as a consequence of using higher taxonomic levels or subsets of species, are shown in Table 3. As can be seen, the savings by pooling species into genera range from 15%, in the case of *Laminaria* samples, to 37% in the "*Corallina*" assemblage. The increase in savings using family level is considerable in both cases, but the aggregation to orders does not represent a reduction in the processing effort in the "*Corallina*" assemblage. The savings when the subsets of taxa are considered are much higher in both assemblages, reaching 55% and 84%, respectively, just at species level. With these reduced matrices, aggregation of species at higher taxonomic levels produced an irrelevant benefit, especially in the case of the "*Corallina*" set of samples.

The values of the CB_L index indicate a good costeffectiveness relation for most treatments of both assemblages, reflecting that there is a balance between precision of the results and decrease in the processing effort by using higher taxonomic levels or subsets of taxa (Fig. 9). Thus, as can be



Fig. 4. Second-stage MDS plot showing patterns of relationship in the dissimilarity structure of the various treatments applied (S = species, G = genus, F = family, O = Order, r = reduced matrices of taxa).

seen, just in the case of fourth root transformations the value of CB_L reaches 0.2, except at order level in some cases of "*Laminaria*" assemblage. By using qualitative data, whichever treatment applied, the loss of information is not compensated by the savings in the processing effort (CB_L > 0.5). Finally, It must be pointed out that, according to this index, a slight decrease in the cost effectiveness is produced from genus (or subset of species) to order, and from raw to fourth root transformed data. These trends are similar using the full data sets or the subsets of taxa with the highest biomass.

4. Discussion

The results of this study support the use of higher taxonomic levels for the assessment of macroalgae assemblages. It has also been found that the loss of information if just representative species are identified does not alter to a great extent the results obtained with the full data set of taxa. Moreover, the results confirm that the type of transformation used introduces major changes in the multivariate pattern of the samples and to a greater extent than the taxonomic level at which organisms are identified.

Thus, the Spearman rank correlations obtained among the species similarity matrices and higher taxa were very high in most cases, although the *r*-values diminished from genera to orders. Only at the order level major changes were detected in the "*Laminaria*" assemblage, whereas no such differences were detected for the "*Corallina*" community. This can be explained by the fact that in the "*Laminaria*" assemblage 30 families are aggregated into 18 orders, while in the "*Corallina*" correlations obtained are not surprising, since most of the genera, families or orders only include 1 or 2 taxonomic units.

The robustness of the taxonomic aggregation depends on the type of transformation used, and the *r*-values diminish as the severity of the transformations becomes higher (from raw to qualitative data). Nonetheless, only minor differences were found among non-, square root and log transformed data. Fourth root transformed data produced somewhat higher drops in correlations, while the loss of information using presence-absence data was noteworthy. This pattern can be explained by the dominance of structural species (Corallina elongata or Laminaria ochroleuca) and the low biomass that most of the accompanying species presented. If the transformation is mild, the few dominant species play an important role in the patterns of similarity obtained among samples, and this pattern is easily retained at higher taxonomic levels. With stronger transformations, as fourth root, the differential effect of dominant species in relation to rare species decreases. Obviously, all taxa become equally important using presence/ absence data. That is to say, that as the severity of transformation increases, the distribution of biomass becomes less important and the taxonomic composition becomes more relevant in the results of multivariate analysis.

According to our results, the underlying patterns related to the environmental factors (wave exposure and depth) identified by the MDS ordination of the samples were retained at most taxonomic levels but the similarity among patterns decreased from genera to orders. Moreover, in general terms, the divergences found among different taxonomic levels were lower than those derived from the types of transformations applied to the data. The MDS plot based on fourth root transformation differed mostly from the plot based on raw data (non-transformed) and, surprisingly, was most similar to the one based on presence/absence data. In fact, the highest values of R in the ANOSIM test were obtained with fourth root transformation and presence/absence data for both communities, despite low correlations between the respective matrices. As mentioned before, this fact could be explained by the differences among samples located in different environmental conditions, which are determined to a great extent by changes in composition rather than changes in the relative biomass of the dominant species. Finally, it must be stated that if fourth root transformations are used, the R values diminish aggregating species in higher taxa, whereas no such loss of information occurs with the remaining transformations,



Fig. 5. MDS plots of "Laminaria" samples obtained from the full data sets and from the subsets of species (biomass higher than 0.5 g dw m^{-2} in some sample), using different transformations and taxonomic levels.

at least in most cases. The most relevant result obtained in the current study is that the effect of data transformation is greater than that of species aggregation at higher taxonomic levels. This result is consistent with the conclusions of previous studies regarding the robustness of taxonomic sufficiency in rocky bottoms (Smith and Simpson, 1993; Juanes and Canteras, 1995; Urkiaga-Alberdi et al., 1999; Pagola-Carte et al., 2002) and the effects of data transformations in the results of multivariate analyses (Olsgard et al., 1997, 1998; Anderson et al., 2005).

The distribution of taxonomic units at higher taxa levels also explains the high correlations obtained among the similarity matrices derived from the full data sets and those constructed using a reduced number of taxa (10 and 30 species in "*Corallina*" and "*Laminaria*", respectively). Thus, as mentioned before, noticeable differences were only produced using fourth root transformed data, while very high divergences appeared when considering the qualitative approach. This means that if species with low biomass are removed, the correlations will remain high if the severity of the transformation is mild, since the role of the dominant species will be similar. Conversely, with stronger transformations, the multivariate pattern changes to a greater extent because the dominant species lose weight and the compositional features, including rare species, become more relevant. These general patterns can be visualized in the second-stage MDS. Thus, we observed that distance among similarity matrices derived from full or reduced data sets only become substantial with fourth root and presence/absence transformed data.

Regarding the MDS plots obtained from a reduced number of taxa, the intertidal community ("*Corallina*") retained a pattern related to wave exposure, while in the subtidal



Fig. 6. MDS plots of "*Corallina*" samples obtained from the full data sets and from the subsets of species (biomass higher than 0.5 g dw m^{-2} in some sample), using different transformations and taxonomic levels.

("*Laminaria*") this was not as clear. Nonetheless, the results of the ANOSIM in both assemblages were similar to those obtained with the full data sets using transformations such as square root or $\log(x + 1)$. In addition, the robustness of the aggregation seems to be very high in most cases, except at order level in the "*Laminaria*" set of samples.

A question arises about if these patterns of taxa distribution are similar in other communities. In the case on the "*Corallina*" assemblage, the assessment of any intertidal area in the Atlantic shore has to include this community, given that constitute an almost continuous fringe in the mean intertidal and comprises a great proportion of the total richness of these areas. More uncertain is the case of the subtidal assemblage, since the variety of communities is higher. Nonetheless, we have take into account that shallow bottoms in these coastal areas are usually characterized by the dominance in biomass of a few structural species (p.e. *Gelidium sesquipedale*, *Plocamium cartilagineum, Cystoseira baccata, Himanthalia elongata*), and a low biomass of the remaining species. As well, it should be noticed that much of the accompanying species are common to the different communities, although the relative biomass changes accordingly to the environmental conditions. Therefore, it is expected that the results would not differ in a great extent including other assemblages or applying a different approach, at least in most of the cases.

As mentioned above, the use of genera or families seems to be a good alternative to the use of species for the assessment of macroalgal assemblages. In both communities, the identification at family level seems to be the better option, taking into account the reduction in the number of taxa to be identified (40% in "*Laminaria*", 34% in "*Corallina*"). In addition, when selecting a specific taxonomic level we have also to consider the difficulties of taxonomical identification of the taxa and their frequency of appearance in the



Fig. 7. Results of the ANOSIM performed among samples of the "*Corallina*" assemblage at semi-exposed and exposed sites, using the full data set and subsets of taxa (biomass higher than 0.5 g dw m^{-2} in some sample), at different taxonomic levels and types of transformation.

assemblage analyzed. For example, 35% of the identified species in the "*Corallina*" samples belonged to just 4 genera (*Callithamnion, Ceramium, Polysiphonia* and *Cladophora*). Their identification to species level requires experience and laborious preprocessing tasks (microscopic slide preparation, cuttings of thalli), whereas the identification at the genus level is relatively easy and does not require the skills of an expert. In such cases, the identification at genus level instead of species level will produce an obvious save in time for processing and identification.

Likewise, the use of representative selected taxa appears to be a suitable approach, taking into account the balance between precision and processing effort. In terms of taxa to be identified, the benefit derived from considering the full data set at family level is similar to that of using subsets of species and genera (55–60% and 84% in "*Laminaria*" and "*Corallina*", respectively). However, the use of conspicuous taxa has obviously an important advantage, given that allows their identification "in situ" in most cases, since the skills needed to identify most taxa in the field are easily acquired (Ballesteros et al., 2007). By using this approach one doesn't need to collect and process samples in the laboratory, a costly task that constitutes a major constraint in many studies. The benefit increases notably in the case of the subtidal habitat, where diving and meteorological conditions may restrict surveys on macroalgae assemblages.



Fig. 8. Results of the ANOSIM performed among samples of the "*Laminaria*" assemblage located at sites below and above 20 m depth, using the full data set and subsets of taxa (biomass higher than 0.5 g dw m^{-2} in some sample), at different taxonomic levels and types of transformation.

Table 3 Cost saved by aggregating species to higher taxonomic levels and using subsets of taxa (biomass higher than 0.5 g dw m^{-2} in some sample), according to

| are meaned proposed by remain a | "Laminaria" | "Corallina" | |
|---------------------------------|-------------|-------------|--|
| Species to genus | 15% | 37% | |
| Species to genus | 55% | 71% | |
| Species to order | 73% | 78% | |
| Species to subset of species | 55% | 84% | |
| Species to subset of genera | 60% | 84% | |
| Species to subset of families | 72% | 86% | |
| Species to subset of orders | 81% | 87% | |

In conclusion, the use of the most conspicuous taxa (species, genera and even families), which are identifiable "in situ" in most cases, would lead to an acceptable approach for the assessment of macroalgae assemblages by saving sampling, processing and identification time. Similar approaches have been proposed by Wells et al. (2007) and Juanes et al. (in press) for the assessment of the ecological status of macroalgae communities of North East Atlantic coastal waters, following the requirements of the WFD. These authors consider the Reduced Species Lists which include only the macroalgae that contribute most significantly to the overall composition of the rocky shores under study. Both proposals suggest low level of taxonomic expertise and familiarization with few algal species. Also, Panayotidis et al. (2004) suggested that taxonomic effort could be limited to the most abundant taxa for the needs of WFD implementation.

However, problems arise regarding the quantification of biomass, given that, as the results of this study show, the use of a qualitative approach may give a misleading picture of the existing macroalgae communities. Alternatively, a semi-quantitative evaluation, considering parameters such as relative coverage for estimating the abundance of species, may give a good approximation of the real structure of the assemblage (Boudouresque, 1971; Dethier et al., 1993; Pagola-Carte et al., 2002; Juanes et al., in press). In fact, the use of untransformed data derived from a semi-quantitative scale of abundance at 5 or 6 levels, is roughly equivalent to the performance of fourth root transformation of biomass data (Clarke and Warwick, 2001). Many authors advise the use of intermediate transformations, as square root or fourth root, for the multivariate analysis of benthic communities (Ferraro and Cole, 1995; Olsgard et al., 1997; Clarke and Warwick, 2001). In the case of macroalgae, the use of fourth root seems to be appropriate, due to the high biomass of structural species and the dominance of a few ones. Thus, the relative coverage estimation procedure proposed by indices such as the CFR (Juanes et al., in press) would represent an acceptable correspondence with a quantitative study based on multivariate analysis of fourth root transformed biomass data.

On the other hand, it should be considered that in the current study we have analyzed natural environmental gradients instead of pollution gradients. So, it is expected that the robustness of the analysis performed would be higher if changes in community structure were due to anthropogenic pressures (Ferraro and Cole, 1990, 1995; Olsgard et al., 1998). Macroalge, as sessile organisms, are good indicators of the existence of organic enrichment produced by sewage discharges (Murray and Littler, 1978; Fairweather, 1990; Orfanidis et al., 2001). In this situation, it is expected that an increase of opportunistic species will occur, producing a shift in the relative abundance of other species (Murray and Littler, 1978). In polluted sites, the development of opportunistic species such as Ulva sp., Enteromorpha sp., Ectocarpaceae or Ceramiaceae, is common, as well as changes in the relative proportion of red, brown and green seaweeds (Murray and Littler, 1978; Gorostiaga and Díez, 1996; Díez et al., 1999; Orfanidis et al., 2001; Wells et al., 2007).



Fig. 9. Values of the CB_L index aggregating species to higher taxonomic levels and using subsets of taxa with a biomass higher than 0.5 g dw m⁻².

Thus, the detection of changes due to anthropogenic activities, as stated by the WFD, would be possible using higher taxonomic levels than species, identifying the most conspicuous ones and applying a semiquantitative approach. For example, the progressive replacement of late successional species such as Corallina elongata by Chondria coerulescens, Caulacanthus ustulatus and Geranium pusillum from slightly to severe polluted areas, as described by Díez et al. (1999) in areas similar to the one analyzed in this study, will be easy to detect with a semiquantitative methodological approach. In relation with this, recent proposed indices consider parameters such as the proportion of opportunistic species (CFR, Juanes et al., in press), the sensitivity of communities in relation with environmental stress (CARLIT, Ballesteros et al., 2007) or the proportion of Ecological Status Groups (ESGs) which represents alternative ecological status from the pristine status, with late-successional species, to the degraded status, dominated by opportunistic species (EEI, Orfanidis et al., 2001, 2003; RSL, Wells et al., 2007).

Therefore, for the implementation of surveillance or operational monitoring, as required by the WFD, it would be suitable to carry out low-budget monitoring programs, as suggested by Panayotidis et al. (2004), by applying a semiquantitative approach. This approach would allow both to assess the ecological status of macroalgae in a straightforward and costeffective way and to characterize wider areas than those possible to assess by means of sample collection (Panayotidis et al., 2004; Ballesteros et al., 2007; Juanes et al., in press). We agree with these authors in that very precise and expensive quantitative studies, in which the information is constrained to punctual samples, produce an incomplete picture of a specific waterbody's macroalgal ecological status. As outlined by Diaz et al. (2004), without a broad-scale mapping of macroalgae distribution patterns it is not possible to undertake their assessment. Therefore, the possible loss of information derived from the application of a semiquantitative sampling procedure is lower than that produced by the blind picture that traditional sampling and specific composition determination in the laboratory have produced, making it difficult to assess the ecological status at water body level, as required by the WFD. This approach is supported by most of the indices proposed for macroalgal assessment, i.e.: RSL (Wells et al., 2007), CARLIT (Ballesteros et al., 2007), or CFR (Juanes et al., in press) which have proved useful in different types of habitats.

Furthermore, as outlined by Juanes et al. (in press), the semiquantitative approach would be suitable for the recognition and characterization of "coastal reef habitats", a habitat type that has been designated of Community Interest and included in the Habitats Directive 92/43/EEC. The implementation of this Directive requires the obtention of information regarding the distribution of macroalgae communities colonizing coastal reefs in a broader scale than those possible to obtain by taking punctual samples. Without this information it would be unfeasible to identify and designate target sites for conservation and that require special management measures, thus preventing further deterioration of relevant areas.

Moreover, the use of a non-destructive technique, such as the semiquantitative procedure, could be the only option in habitats colonized by sensitive species with slow recovery rates.

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