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Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive

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Abstract

The European Water Framework Directive (WFD) establishes a framework for the protection and improvement of estuarine and coastal waters, trying to achieve 'good surface water status at the latest 15 years after the date of entry into force of this Directive'. One of the biological elements that should be analysed is the benthos and, as such, the WFD normative definitions describe the aspects of the benthic communities that must be included in the ecological status assessment of a water body. Therefore, it is essential to include, in the assessment, the different metrics that address those parameters identified in the normative definitions for each of the ecological status classes. In this contribution the use of the AMBI, richness and diversity, combined with the use, in a further development, of factor analysis together with discriminant analysis, is presented as an objective tool (named here M-AMBI) in assessing ecological quality status. This assessment requires previous classification of water bodies and typologies, together with the definition of reference conditions; this is undertaken in this contribution using historical data, expert judgement and multivariate analysis. The study has been undertaken by examining changes in benthic communities in the Basque Country, over the last decade, as a case-study, to demonstrate the accuracy and potential of these methodologies.

Keywords: Water Framework Directive; Ecological Quality Status; Macrozoobenthos; AMBI; Richness; Diversity; Factor analysis; Discriminant analysis

1. Introduction

The European Water Framework Directive (WFD; 2000/60/EC) establishes a framework for the protection and improvement, amongst others, of estuarine and coastal waters; its final objective is to achieve at least 'Good water status' for all waters, by 2015. The WFD requires member states to assess the ecological quality status (EcoQS) of water bodies, with a 'water body' being 'a discrete and significant element of surface water such as a lake, a river, a transitional water or a stretch of coastal water'. The suggested hierarchical approach to the identification of surface water bodies includes: (i) the definition of the River Basin District (including freshwater, estuarine and coastal waters); (ii) the division of surface waters into one of six surface water categories (i.e. rivers, lakes, transitional waters, coastal waters, artificial and heavily modified water bodies); (iii) the sub-division of surface water categories into types, then assigning the surface waters to one type; and (iv) the sub-division of a water body of one type into smaller water bodies, according to pressures and resulting impacts (for details, see Vincent et al., 2002; Borja et al., 2004a,b, 2006a; Heiskanen et al., 2004; Borja, 2005). Afterwards, it is necessary to determine reference conditions for each of the typologies and, likewise, to assess the EcoQS for each of the water bodies. This assessment will be based upon the status of the biological, hydromorphological and physico-chemical quality elements, by comparing data obtained from monitoring networks with the reference

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(undisturbed) conditions, then deriving an Ecological Quality Ratio (EQR). The ratio shall be expressed as a numerical value between zero and one, with 'High status' represented by values close to one and 'Bad status' by values close to zero. In coastal and transitional (which include the estuaries) waters, the biological elements to be considered are phytoplankton, macroalgae, benthos and fishes (with the latter only in transitional waters).

Recently, some methodological approaches in implementing such a complex Directive have been undertaken in Europe, including integrative methodologies (Henocque and Andral, 2003; Borja et al., 2004b; Casazza et al., 2004), with others focused upon some of the elements, such as the physico-chemical elements (Casazza et al., 2002; Nielsen et al., 2003; Andersen et al., 2004; Bald et al., 2005), phytoplankton (Ifremer, in Vincent et al., 2002; Borja et al., 2004b), or macroalgae (Orfanidis et al., 2001, 2003; Swedish method, in Vincent et al., 2002; Panayotidis et al., 2004; Borja et al., 2004b).

In the particular case of benthos, the WFD normative definitions describe the aspects of the benthic community that must be included in the EcoQS assessment of a water body. Therefore, it is essential that any proposed classification scheme for WFD assessment include indices (metrics) that address those parameters identified in the normative definitions for each of the five ecological status classes i.e. 'High', 'Good', 'Moderate', 'Poor' and 'Bad' (see Vincent et al., 2002; Borja et al., 2004b). Hence, the main terms to be addressed by a benthic invertebrate classification scheme for WFD are: 'the level of diversity and abundance of invertebrate *taxa*'; and the proportion of 'disturbance-sensitive *taxa*'. Following these criteria, several indices and approaches have been proposed in assessing WFD EcoQS for the benthic component (Borja et al., 2000, 2003a, 2004b,d; Simboura and Zenetos, 2002; Rosenberg et al., 2004). All of these have focused upon the proportion of disturbance-sensitive *taxa*, being currently the AMBI index (Borja et al., 2000) one of the most widely used in European countries and WFD methodologies Intercalibration Working Groups (Borja et al., 2006c). These methodologies have been compared elsewhere (Chainho et al., 2006; Labrune et al., 2006; Quintino et al., 2006).

However, the use of these indices, together with the remainder of the community structural parameters determined by the WFD, is less extended. Hence, in a first approach, Borja et al. (2003b) proposed the use of AMBI, richness and diversity. In a further development, Borja et al. (2004b) suggested the use of multivariate analysis techniques such as the factor analysis (FA) with the principal component analysis as extraction method (see Meglen, 1992; Vega et al., 1998; Hair et al., 1999) as an objective tool in assessing the EQR. The use of FA, for environmental impact assessment studies, was developed initially by Algarra and Niell (1985) and Niell et al. (1988). Similar methodologies have been developed by Smith et al. (1993, 1999, 2001), Bald et al. (1999, 2001) and Gibson et al. (2000) in the determination of human impact on benthic and fish communities.

Recently, Bald et al. (2005) applied the FA in the assessment of physico-chemical status, according to the WFD, solving some of the problems underlined by Borja et al. (2004b) when using this particular methodology. However, Bald et al. (2005) proposed the study of the response of the FA when new data, or sampling stations, are incorporated into a WFD monitoring network. This study was suggested because the position of the sampling stations, within the new three-dimensional space (as defined by the FA), can change when new data are incorporated. Consequently, the biological status of these sampling stations could be different, in comparison with the assessment obtained without new data in the FA. In order to avoid this effect, Bald (2005) and Bald et al. (2005) propose the use of statistical multivariate methods, such as discriminant analysis (DA), together with FA. This methodology has been used also in assessing benthic quality by Paul et al. (2001). Although FA analysis is used broadly in water quality assessment, this is not the case for DA. One of the few applications can be found in Alberto et al. (2001).

The main objective of this contribution is to demonstrate the usefulness of DA techniques, together with FA, and the accuracy and potential of these methodologies, in determining the benthic status, according to the WFD. Likewise, to solve the problems outlined above and, as an improvement of previous contributions of our research team, this study utilises the changes in benthic communities in the Basque Country, over the last decade, as a case-study.

2. Material and methods

2.1. Selection of structural parameters

The Department of Land Action and Environment of the Basque Government, by means of the *Littoral Water Quality Monitoring and Control Network* (hereafter, LQM), has monitored Basque coastal and estuarine water quality since 1995 (Borja et al., 2005). This network comprises the analyses of both physico-chemical (in water, sediment and biota) and biological elements (phytoplankton, macroalgae, benthos and fishes). The LQM series data includes 32 coastal and estuarine stations, sampled from 1995 to 2004, with 19 more since 2002 (Fig. 1). These data have been used in the case-study presented in this contribution.

Soft-bottom macrobenthic communities are sampled annually, always in winter (see sampling methods in Borja et al., 2003b). The parameters which are determined in the abovementioned framework include density, biomass, species richness (number of *taxa*), Shannon Wiener diversity index, Pielou's evenness, maximum diversity and AMBI (Borja et al., 2000). Guidelines derived from Borja and Muxika (2005) are used in the calculation of the AMBI, using the species list of October 2005.

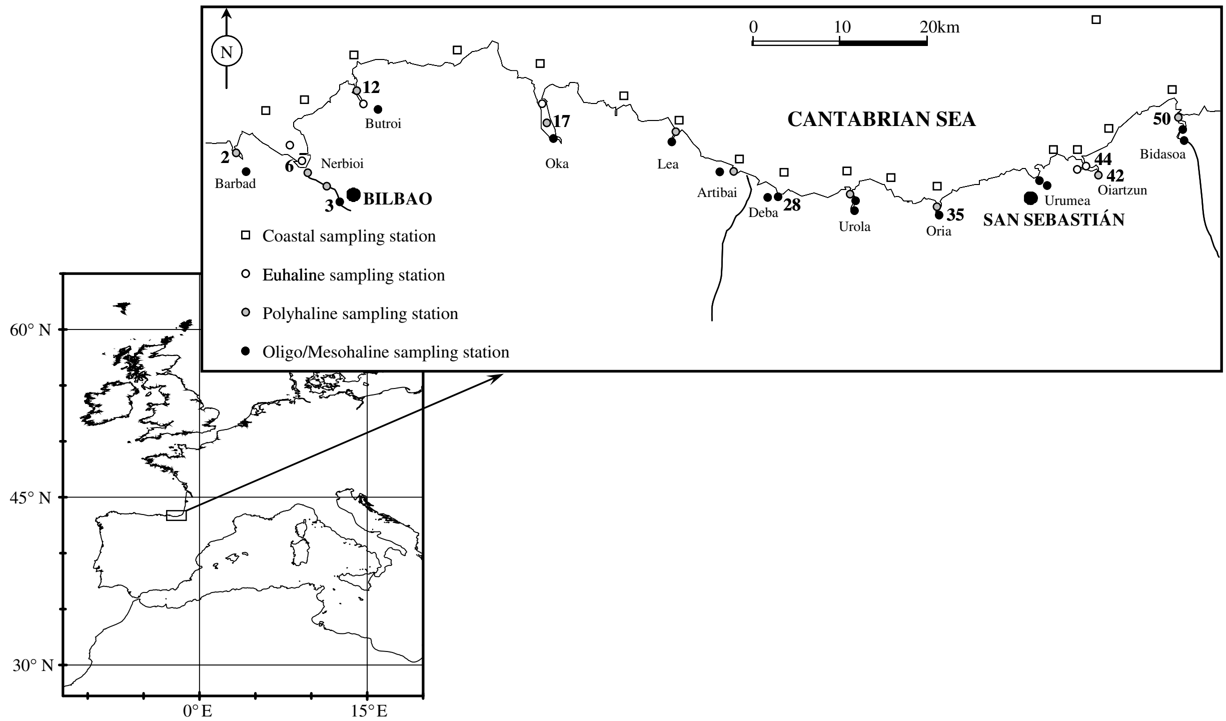

Fig. 1. LQM sampling stations and typologies. The stations selected to analyse trends are shown with the same numbers as in Bald et al. (2005). The Deba and Urumea estuaries are assigned to Type I (small river-dominated estuaries). The Barbadun, Butroi, Oka, Lea, Artibai, Urola and Oria estuaries are assigned to Type II (estuaries with wide intertidal flats). The Nerbioi, Oiartzun and Bidasoa are assigned to Type III (estuaries with wide subtidal areas). The coastal stations are assigned to Type IV (full marine exposed coast).

As mentioned above, the main terms to be addressed by a benthic invertebrate classification scheme for WFD are: diversity; abundance of invertebrate *taxa* (it is not clear if WFD refers to density or/and richness); and the proportion of 'disturbance-sensitive *taxa*'. In this contribution, density, biomass, richness, Shannon Wiener index and AMBI (as an index which determines the proportion of disturbance-sensitive *taxa*) have been selected; this is in order to determine the parameters which better define the EcoQS of the water bodies studied. Of course, the selection and use of other metrics and indices are also valid (see Prior et al., 2004 or Rosenberg et al., 2004), if they are intercalibrated with other methodologies (see Borja et al., 2006c).

2.2. Water bodies and typologies

The Basque coastal and transitional water typologies have been established by Borja et al. (2004a,b) as: (i) small river-dominated estuaries (Type I); (ii) estuaries with extensive intertidal flats (Type II); (iii) estuaries with extensive sub-tidal areas (Type III); and (iv) full marine exposed coasts (Type IV) (see Fig. 1). At present, 14 transitional and 4 coastal water bodies have been determined in the Basque Country, following the study of pressures and impacts, *sensu* WFD (Borja et al., 2004a, 2006a).

The approach developed by Borja et al. (2004b) considers a water body (e.g. an estuary) as an entity; however, this produces some problems in establishing reference conditions for the whole of the water body (Borja et al., 2003b). In order to fit the classification of the various water bodies to their hydrographical properties, each of the water bodies was split into different stretches, using the salinity gradient as a characterisation factor (see Bald et al., 2005).

2.3. Deriving reference conditions

The reference condition for a water body type is a description of the biological elements which corresponds totally, or nearly totally, to undisturbed (= pristine) conditions, i.e. with no, or with only a very minor, impact from human activities (as mentioned by the WFD). The objective of setting reference condition standards is to enable the assessment of the biological quality, against these standards. Type-specific reference conditions must summarise the range of possibilities and values for the biological quality elements, over periods of time and across the geographical extent of the type (Vincent et al., 2002).

The WFD identifies four options for deriving reference conditions: (i) comparison with an existing 'pristine'/undisturbed site (or a site with very minor disturbance); (ii) historical data and information; (iii) models; or (iv) expert judgement. Jonge et al. (2006) discuss the adequacy of data and information for purposes other than trend analysis and compliance. Besides, Borja et al. (2004b) have stated that one of the problems in deriving reference conditions in some European regions arises from the absence of unimpacted areas. This is the case for the Basque Country, in which all the estuaries have been historically impacted by human activities, especially over the last 150 years (Cearreta et al., 2004; Borja et al., 2004b, 2006a). Moreover, this region does not have any pre-industrial historical data; hence, the use of 'virtual' reference locations (as defined and proposed in Borja et al., 2004b), as an 'expert judgement' approach, requires consideration. The use of 'virtual' reference locations has been used successfully in the case of physico-chemical elements (Bald et al., 2005).

Each of the defined typologies has a main associated benthic community (see a similar approach in Perus et al., 2004), depending also upon the salinity stretches determined and the associated habitats (Table 1). These benthic communities have been described in detail by Borja et al. (2004c).

Following the approach described in Bald et al. (2005), two levels of reference conditions were constructed for each stretch, i.e. those representatives of 'High' and 'Bad EcoQS'. 'High' reference values for each of the structural parameters and communities were selected from Borja et al. (2004c). In the case of AMBI, the index is equal to zero when all the species in a sampling station are assigned to Ecological Group I (EG I), i.e. only sensitive species are present (for details, see Borja et al., 2000, 2003a, Muxika et al., 2005). In some naturally-enriched areas, such as estuaries, other EGs are present, i.e. EG II (composed by indifferent species) and EG III (tolerant species). In this particular case, the value for 'High' biological status cannot be zero, because it never will be reached within the estuary. Besides, the WFD states that *all* sensitive species, but *any* indicator species (EG IV and V), should be present in a water body to reach a 'High EcoQS'. Hence, in order to determine high reference AMBI values, average densities of species, for the period 1995–2003, were obtained for each of the typologies monitored in the LQM. Subsequently, opportunistic species (EG IV and V) were removed from the species list and the AMBI was derived for each typology, following the guidelines in Borja and Muxika (2005).

Conversely, 'Bad' reference conditions were selected from azoic sediments. Hence, all the structural parameters are considered as zero value and the AMBI is equal to seven.

2.4. Biological status assessment

Using data from the 1995–2003 series, a FA was used for the determination of the EQR for each of the typologies, with their corresponding references grouped in the different stretches (Borja et al., 2003b). Data were standardised, by subtracting the mean and dividing by the standard deviation in order to achieve a normal distribution of the data. The FA solution was rotated (using the Varimax rotation method) in order to make easier the interpretation of the analysis results (see Hair et al., 1999). The application of such methodology, including the derivation of the EQR and EcoQS, can be consulted in Bald et al. (2005).

The threshold values for the EcoQS classification (EQR determination), based upon the REFCOND (2003), were: 'High', >0.82; 'Good', 0.62–0.82; 'Moderate', 0.41–0.61; 'Poor', 0.20–0.40; and 'Bad', <0.20. These values accomplished the WFD requirements and the recommendations of Borja et al. (2004b), and were selected until further intercalibration of the methodology (see Borja et al., 2006c).

Based upon the FA, the 1995–2003 period was used for the determination of the discriminant functions, by means of a DA for each of the salinity stretches; then, those functions were applied to the 2004 data set. DA is considered appropriate when data can be classified into two or more groups and when one or more functions of quantitative measurements, which can help in discriminating among the known groups, are required (Paul et al., 2001).

The objective of this analysis is to provide a method for predicting which group a new case is most likely to fall into, depending upon the different quantitative values of the selected structural parameters (Bald, 2005). The main properties of these discriminant functions are: (i) they are constant; (ii) they do not change over time; and (iii) they do not change with new data addition (Bald, 2005; Bald et al., 2005). It is considered one of most appropriate statistical methods when the dependent variable is qualitative, such as the EcoQS, and the independent variables are quantitative, such as the selected structural parameters (Hair et al., 1999). The concept of DA involves forming linear combinations of independent (predictor) variables, named discriminant functions, which become the basis for group classifications. These functions are as shown below:

$$Z_{jk} = a + W_1X_{1k} + W_2X_{2k} + W_3X_{3k} + \cdots W_nX_{nk}$$

where Z_{jk} = discriminant score of the discriminant function j for the object k, a = constant, W_i = discriminant weight for the independent variable i, X_{ik} = independent variable i for the object k.

Table 1
Main benthic communities associated to each of the water body typologies, in the Basque Country

Salinity stretches	Type I	Type II	Type III	Type IV
Oligo-/mesohaline	*C. edule–S. plana*	*C. edule–S. plana*	*C. edule–S. plana*	–
Polyhaline	–	*V. fasciata*	*V. fasciata*	–
Euhaline	–	*A. alba*	*A. alba*	*T. tenuis–V. fasciata*

Note that only two of the estuaries classified as Type II (Butroi and Oka) maintain an *Abra alba* community. Key: Type I = small river-dominated estuaries; Type II = estuaries with extensive intertidal flats; Type III = estuaries with extensive subtidal areas; and Type IV = full marine exposed coast.

DA is appropriate for testing the hypothesis that the group means, for two or more groups, are equal. Each independent variable is multiplied by its corresponding weight, then the products are added together; this results in a single composite discriminant score for each individual in the analysis. Averaging the scores derives a centroid group. If the analysis involves two groups, there are two centroids; in three groups, there are three centroids; and so on. Comparing the centroids shows the distance of the groups along the dimension that is being tested. The objectives for applying DA include:

- determining if there are statistically significant differences among two or more groups;
- establishing procedures for classifying stations into groups; and
- determining which independent variables account for most of the difference in two or more groups.

The outputs of the DA are as many discriminant functions as groups have been established. These functions are used to calculate a 'probability' for a given station of belonging to each of the groups.

Some of the sampling stations were selected to look for possible trends, or temporal changes, in the biological status assessment and to compare these changes with those detected by Bald et al. (2005). Trends were analysed by means of Spearman rank correlations between the EQRs and time (in years). Distinct changes were analysed by 'before–after' comparisons (two-sample comparison of means), when any change in pressures was known in the water body to which the sampling station was allocated.

The statistical analyses were carried out using Statgraphics® Plus 5.0.

2.5. Validation of the method

Whatever method is used, there will always be problems in establishing the EcoQS of some stations, as outlined below:

- Stations with high hydrodynamism (i.e. in highly exposed coasts) present usually low structural parameter values (such as richness and diversity) and, sometimes, high AMBI. These stations can be classified in terms of 'Poor' or 'Bad EcoQS', although they are not subjected to anthropogenic impacts, but to natural stress.
- In some locations where recolonisation processes are occurring, there could be many differences among the structural parameter values, misclassifying the station i.e. sudden increases in richness, diversity or/and abundance (Borja et al., 2006b).

In order to avoid such misclassifications, different investigators assessed the stations EcoQS on the basis of expert judgement (see Prior et al., 2004). In this contribution, the composition of each of the sampling stations, together with the structural parameters, was sent to three experts; these assessed the quality of each of the samples, into the five WFD levels, based upon their own experience. This was a subjective process in which each of the experts evaluated the general quality of the area, based upon their knowledge of the locations and the knowledge of the benthic communities' composition and structure. In order to determine a unique EcoQS for each of the samples, a relative rating (5, 4, 3, 2, and 1, respectively) has been allocated to each of the abovementioned five levels. Subsequently, a mean value was calculated: (i) 'Bad status' (values <1.5); (ii) 'Poor status' (1.6–2.5); (iii) 'Moderate status' (2.6–3.5); (iv) 'Good status' (3.6–4.5); and (v) 'High status' (>4.6). To analyse the agreement, in assessing the quality status both by DA and by expert judgement, a Kappa analysis was undertaken (Cohen, 1960; Landis and Koch, 1977). The level of agreement between both methods was established, based upon the equivalence table from Monserud and Leemans (1992). As the importance of misclassification is not the same between close categories (e.g. between 'High' and 'Good', or 'Poor' and 'Bad') as between further categories (e.g. between 'High' and 'Moderate', or 'High' and 'Bad'), Fleiss-Cohen weights were applied to the analysis (Fleiss and Cohen, 1973).

3. Results

3.1. Selection of structural parameters

The results of the FA (Fig. 2), carried out with the five structural parameters and all the sampling stations, showed that the first three factors explained 87% of total variance. The first factor presented an eigenvalue of 2.1. The eigenvalue for the second factor was 1.2 and it was 1.0 for the third factor. The first factor was positively related mainly to richness and Shannon Wiener diversity, with the second factor being negatively related mainly to AMBI, with the third one to density (positively).

Following the FA, it was determined that some structural parameters, such as biomass and density, show many inconsistencies in relation to environmental quality, although they explain much of the overall variability. In order to avoid this problem, richness, diversity and AMBI, were selected in the subsequent analysis. This grouping was considered the best combination of metrics, as they were the most important parameters in the first two factors of the factor analysis. In this particular case, relative abundances of sensitive and opportunistic species are included already within the AMBI formula, accomplishing the WFD requirements.

Hence, a new FA was carried out only with Shannon Wiener diversity, richness and AMBI (Fig. 2); this showed an eigenvalue of 2.1 for the first factor, 0.7 for the second factor and 0.2 for the third one. The first factor was positively related mainly to richness; the second one was negatively related to AMBI, with the third being positively related to Shannon Wiener diversity.

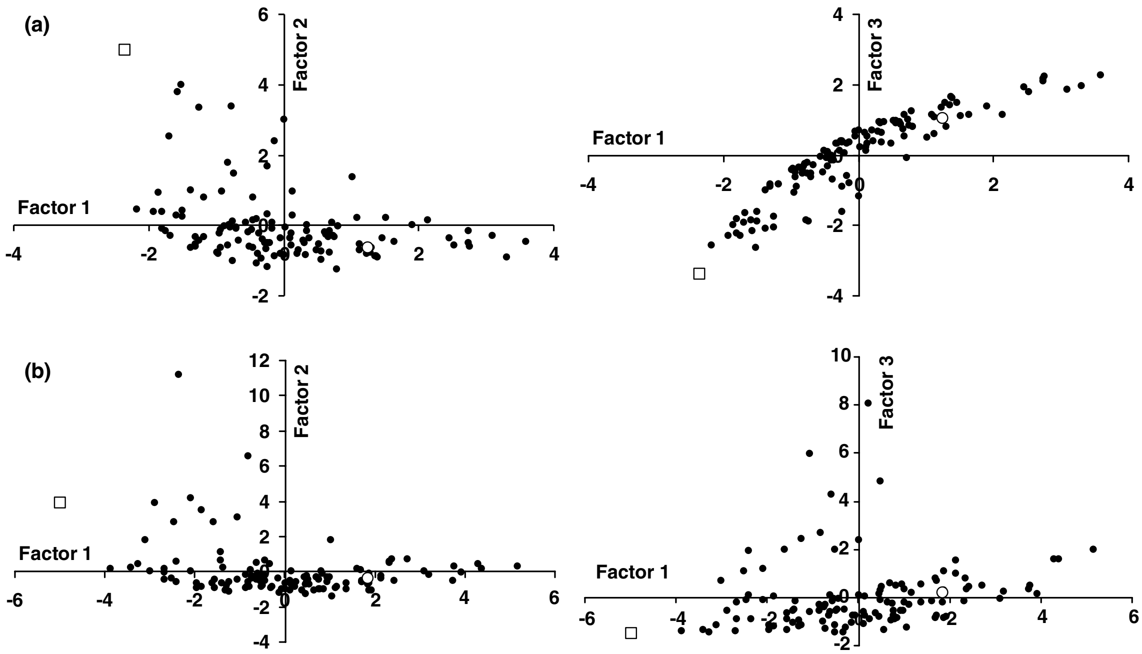

Fig. 2. Distribution of the LQM sampling stations (1995–2003 sampling period), within the new three-dimensional space defined by the FA, relating to the first and second factors (left) and to the first and third factors (right). Based upon: (a) AMBI, richness and Shannon Wiener diversity index; and (b) AMBI, biomass, density, richness and Shannon Wiener diversity index, calculated both with biomass and density data. Key: (○) 'High EcoQS' reference stations; (□) 'Bad EcoQS' reference stations; (●) real stations.

3.2. Deriving reference conditions

The *Scrobicularia plana–Cerastoderma edule* community is found in the inner and middle part of all of the estuaries (Table 1), normally in muddy sand flat bottoms and well oxygenated waters (Borja et al., 2004c). This community involves a higher abundance of *S. plana* and *C. edule*, being composed by euryhaline species, such as the polychaetes *Nereis diversicolor*, *Streblospio shrubsolii*, *S. benedicti* and *Heteromastus filiformis*, the Oligochaeta, the prosobranch *Hydrobia ulvae*, the bivalve *Ruditapes decussatus* and the crustaceans *Cyathura carinata*, *Carcinus maenas*, *Corophium* sp., *Pachygrapsus marmoratus* and *Ampelisca brevicornis*, amongst others. The reference values for this community, together with associated water body stretches (oligo-/mesohaline), are shown in Table 2.

The *Venus fasciata* community is typical of sandy bottoms, in water depths of 20–40 m (Borja et al., 2004c) (Table 1). The most characteristic species are *V. fasciata*, *Venus casina*, *Chamelea striatula*; *Nephtys cirrosa*, *Urothoe brevicornis*, *Bathyporeia elegans*, *Prionospio steenstrupi*, *Echinocardium cordatum*, *Branchiostoma lanceolatum*, *Spisula subtruncata*, etc. The reference values for this community, together with the associated water body stretches (estuarine euhaline), are listed in Table 2.

The *Abra alba* community appears in permanently submerged estuarine areas (Borja et al., 2004c) (Table 1), in sediments of high organic matter and mud content; generally, it occurs within the middle part of the estuaries. The most common species within this community are the molluscs *A. alba*, *Abra prismatica*, *Corbula gibba* and *Thyasira flexuosa*. Other accompanying species are *Lagis* (= *Pectinaria*) *koreni*, *Mysella bidentata*, *Cerianthus membranaceus*, *Polydora polybranchia*, etc. The reference values for this community, together with associated water body stretches (polyhaline), are listed in Table 2.

The *Tellina tenuis* community appears both in deep estuaries and in the circalittoral area, in mixed sediments dominated by sand and mud (Borja et al., 2004c) (Table 1). The core of the community is represented habitually by *Tellina fabula*, and *T. tenuis*, together with *Nephthys hombergii*, *Spiophanes bombyx*, *Gouldia minima*, *Nucula* sp., *Dentalium dentalis*, *E. cordatum*, *Dispio uncinata*, *N. cirrosa*, *Cumopsis fagei*, *Diogenes pugilator*, *Glycera* sp., etc. The reference values for this community, together with associated water body stretches, are listed in Table 2.

3.3. Assessing the EcoQS

The distribution of the LQM samples in the new three-dimensional space defined by the FA, corresponding to 1995–2003, is shown in Fig. 2. Based upon the same data

Table 2
Reference conditions of 'High EcoQS' for each of the saline stretches

	Oligo-/mesohaline	Polyhaline	Euhaline	Coastal area
S (no. sp.)	13	32	40	42
H' (bit · ind^{-1})	2.5	3.8	3.5	4.0
AMBI	2.8	2.0	2.1	1.0

S = species richness; H' = Shannon Wiener diversity index.

series, and the three biological parameters selected previously, a FA was carried out for each of the stretches (Fig. 3). The eigenvalues and the main parameters included into each factor are shown in Table 3. Richness is the main factor explaining the variability of the first factor within the oligo-/mesohaline stretch and the coastal area, whilst the AMBI is the main factor in the polyhaline and euhaline stretches. The second factor is related with AMBI in the

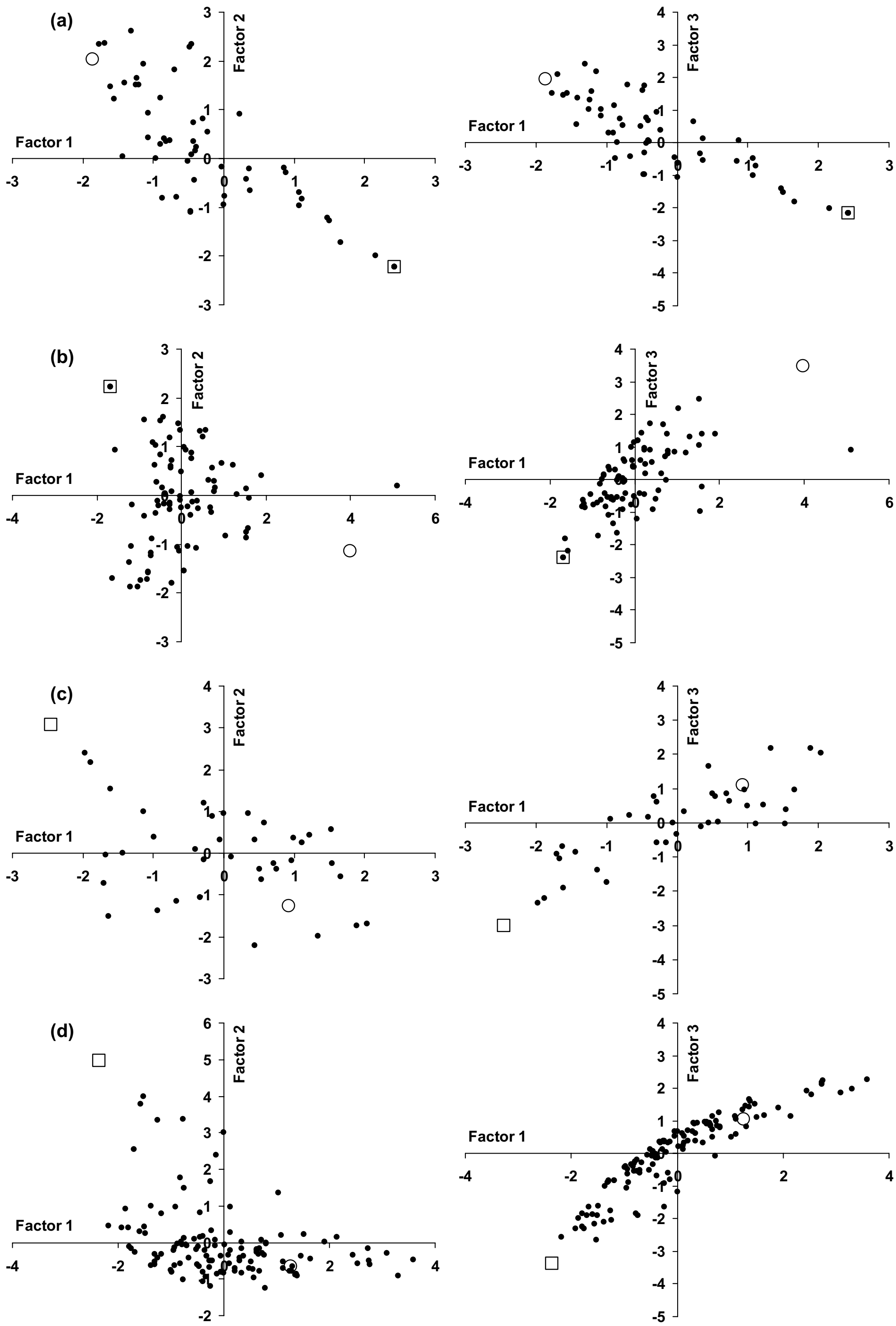

Fig. 3. Distribution of the LQM sampling stations (1995–2003 sampling period) within the new three-dimensional space defined by the FA, relating to the first and second factors (left) and to the first and third factors (right). (a) Oligo- and mesohaline sampling stations; (b) polyhaline sampling stations; (c) euhaline sampling stations; and (d) coastal sampling stations. Key: (○) 'High EcoQS' reference stations; (□) 'Bad EcoQS' reference stations; (●) real stations.

Table 3
Eigenvalues for each of the factors and stretches, derived from FA, together with the main parameter included in of each of the factors (into brackets)

Stretches	1st Factor	2nd Factor	3rd Factor
Oligo/mesohaline	1.9 (richness)	0.9 (AMBI)	0.2 (diversity)
Polyhaline	1.5 (AMBI)	1.1 (richness)	0.5 (diversity)
Euhaline (estuarine)	2.4 (AMBI)	0.5 (richness)	0.1 (diversity)
Euhaline (coastal)	1.8 (richness)	0.9 (AMBI)	0.3 (diversity)

oligo-/mesohaline stretch and the coastal area, being related to richness in polyhaline and euhaline stretches. The third factor is always related to Shannon's diversity (Table 3).

Among the 300 samples analysed, 156 (52%) were classified in 'High' or 'Good EcoQS', and 144 samples did not accomplish the objective of the WFD (Table 5). The highest percentage accomplishing the objective of attaining at least 'Good EcoQS' is reached by the coastal samples (81%). The lowest percentage corresponds to those estuarine samples obtained from stations located in polyhaline stretches (6%).

From the whole of the data set, 10 sampling stations, coinciding with Stations 2, 3, 6, 12, 17, 28, 35, 42, 44 and 50 shown in Bald et al. (2005), have been used in the study of the EQR evolution (Fig. 4).

A general quality improvement has been detected at Stations 3, 28, 42 and 50, from the beginning of the data series (Fig. 4a). Stations 3 and 42 show a progressive, and significant, for $\alpha = 0.1$, improvement (Spearman rank correlation $p = 0.04$ and 0.01, respectively). On the other hand, Station 28 presents significantly higher EQR after 1999, than before (except in 1996) (t-test $p = 0.01$). Station 50 presents higher EQR after 2000, than before (t-test $p = 0.02$).

Conversely, a negative trend is detected at Stations 2, 35 and 44 (Fig. 4b). Stations 2 and 44 show a progressive and slow negative trend (Spearman rank correlation $p = 0.08$ and $p = 0.07$, respectively), whereas Station 35 presents lower EQR after 2000, than before (t-test $p = 0.07$).

Stations 6, 12 and 17 did not show any clear trends throughout the period (Fig. 4c), with minor EQR fluctuations; Station 6 was the most constant (Spearman rank correlation $p > 0.03$).

On the other hand, the DA undertaken for each of the saline stretches (according to the results obtained by means of the FA) resulted in the discriminant function below (1), where a, b, c and K should be replaced by the discriminant coefficients presented in Table 4.

$$\text{EcoQS} = K + a \cdot \text{AMBI} + b \cdot H' + c \cdot S \quad (1)$$

The EcoQS evaluation for 2004, according to those functions, shows that 63% of sampling stations attained at least 'Good EcoQS'. The maximum accomplishment percentage was reached by coastal stations (88%), whereas the minimum percentage was reached, once again, by those sampling stations located in polyhaline stretches (27%) (Table 5).

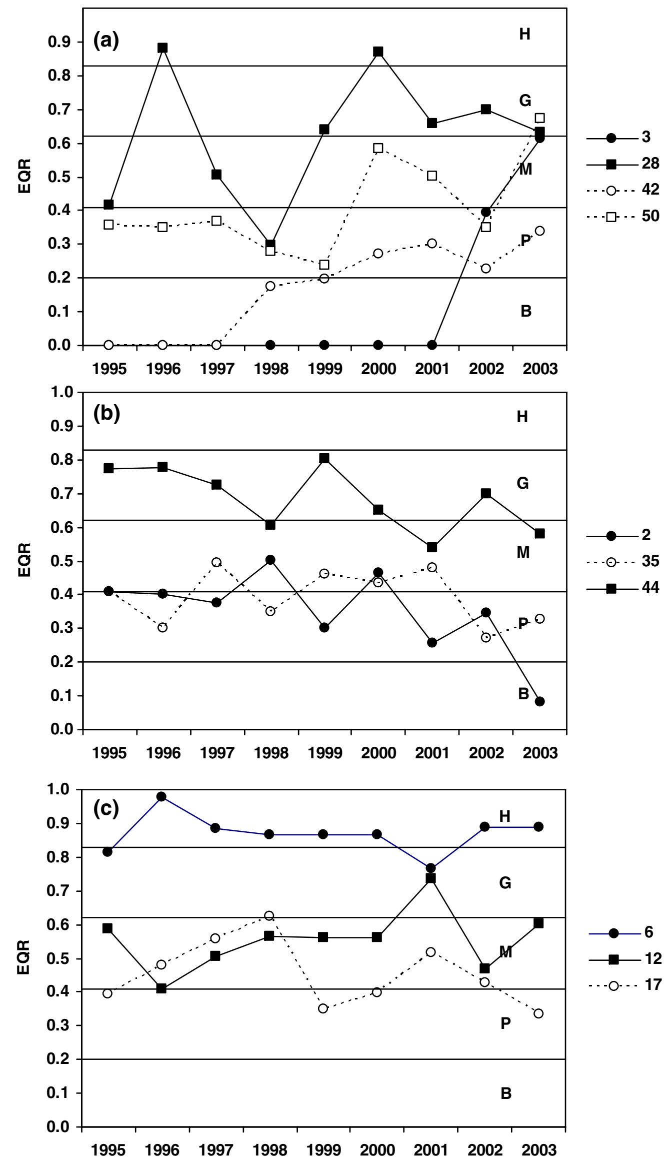

Fig. 4. Trends for some of the sampling stations analysed in this contribution. Note that the selected stations coincide with those in Bald et al. (2005); these are represented in Fig. 1. Key: H = 'High EcoQS'; G = 'Good EcoQS'; M = 'Moderate EcoQS'; P = 'Poor EcoQS'; B = 'Bad EcoQS'.

After applying expert judgement, only 5 of 49 sampling stations (10%) changed their classification. Kappa analysis showed an almost perfect agreement ($K = 0.86$) (see Monserud and Leemans, 1992, for terminology). In terms of typologies, almost perfect agreement was reached for polyhaline ($K = 0.89$) and oligo/meso- and euhaline stretches ($K = 0.90$), whereas the agreement was only moderate for the coastal area ($K = 0.46$) (Table 6).

Expert judgment did not substantially change the number of sampling stations which did not accomplish the WFD objectives (65%); however, it did change the relative proportions between the different stretches. Amongst the coastal sampling stations, up to 94% reached at least 'Good

Table 4
Discriminant coefficients obtained from DA, carried out on the 1995–2003 data series, for each of saline stretches and EcoQS

EcoQS	*a*	*b*	*z*	*K*
Oligo- and mesohaline stretches				
High	−0.915	15.706	3.155	−39.254
Good	3.396	8.678	1.123	−19.542
Moderate	7.103	1.219	−0.151	−15.863
Poor	13.191	−5.237	−1.361	−36.679
Bad	19.105	−13.722	−3.192	−66.911
Polyhaline stretches				
High	−6.093	11.430	2.820	−63.746
Good	−2.107	10.015	0.895	−19.786
Moderate	−0.310	5.571	0.430	−7.198
Poor	2.517	1.795	−0.065	−7.573
Bad	6.573	−4.713	−0.920	−21.491
Euhaline stretches				
High	0.937	8.827	0.158	−22.365
Good	2.559	6.199	0.019	−14.334
Moderate	3.948	3.901	−0.086	−12.137
Poor	5.772	1.750	−0.216	−15.931
Bad	7.804	0.783	−0.342	−26.139
Coastal area				
High	−2.140	6.883	0.050	−13.943
Good	1.699	3.816	−0.063	−6.772
Moderate	8.230	−0.937	−0.073	−12.380
Poor	17.751	−3.497	−0.226	−45.856
Bad	24.221	−7.440	−0.289	−80.593

EcoQS', whereas over 36% of the polyhaline sampling stations achieved the objective of reaching 'Good EcoQS'.

The WFD states that EcoQS has to be assessed for every three year period. For this assessment, average species richness, diversity and AMBI were calculated for the 2002–2004 period. These average values were used in the discriminant functions. For this period, 65.3% of sampling stations reached at least 'Good EcoQS'. All the coastal stations accomplished the WFD, but only 18.2% of the polyhaline stations.

4. Discussion

Macrobenthic communities are considered good indicators of ecosystem health because of their strong link with sediments, which, at the same time, are linked to the water column (Dauer et al., 2000). Hence, benthos shows the real effects of pollution over the communities, being an integrator of the recent pollution history in the sediment and of different kinds of pollutants, which can act synergically: as such, they are a good indicator (Occhipinti-Ambrogi and Forni, 2004).

In the WFD marine quality assessment, based upon benthic communities, there are several key steps for an acceptable approach: (i) an accurate classification of water bodies and typologies; (ii) the selection of reference conditions, for each of the typologies, representative of the absence of anthropogenic influence; (iii) the availability of good classification tools or metrics; and (iv) the suitability of suggested ecological class boundaries. All of these steps are discussed below.

The typology pattern proposed by Borja et al. (2004a) for the Basque Country, together with the approach used in their definition, accomplished with the WFD criteria, and is similar to that defined by other countries (Perus et al., 2004). The Basque estuaries differ in size, morphology or hydrodynamics (Valencia and Franco, 2004); however, they also differ in anthropogenic pressure (Borja et al., 2004a, 2006a), and salinity (Bald et al., 2005), providing a good basis for the final typology and saline stretch determination used in this contribution (this approach is very similar to that of Dauer et al., 2000 and Llansó et al., 2002a, in the US).

Table 5
Number and percentage of samples with 'Bad', 'Poor', 'Moderate', 'Good' and 'High EcoQS', for the 1995–2003, 2004 and 2002–2004 periods

EcoQS	Oligo-/mesohaline		Polyhaline		Euhaline		Coastal area	
	N	%	*N*	%	*N*	%	*N*	%
1995–2003 period								
High	13	22	0	0	12	33	62	51
Good	14	24	5	6	14	39	36	30
Moderate	16	28	32	38	7	19	18	15
Poor	5	9	41	48	1	3	4	3
Bad	10	17	7	8	2	6	1	1
2004								
High	2	13	1	9	2	33	11	65
Good	7	47	2	18	2	33	4	24
Moderate	4	27	6	55	1	17	1	6
Poor	2	13	2	18	0	0	1	6
Bad	0	0	0	0	1	17	0	0
2002–2004 period								
High	3	20	1	9	1	17	10	59
Good	5	33	1	9	4	67	7	41
Moderate	4	27	7	64	0	0	0	0
Poor	3	20	2	18	0	0	0	0
Bad	0	0	0	0	1	17	0	0

Table 6
EcoQS of the 49 sampling stations sampled in 2004, classified both by DA and by expert judgment (for station locations, see Fig. 1)

Saline stretch	Sampling station	Discriminant analysis	Expert judgment
Oligo-/mesohaline stations	1	Good	Good
	3	Moderate	Poor
	10	Good	Good
	16	Moderate	Poor
	21	High	High
	24	Poor	Poor
	27	Moderate	Moderate
	28	Good	Good
	30	Good	Good
	31	Good	Good
	34	Good	Good
	39	Poor	Poor
	40	Moderate	Moderate
	48	Good	Good
	49	High	High
Polyhaline stations	2	Moderate	Moderate
	4	Moderate	Moderate
	5	High	High
	12	Good	Good
	17	Moderate	Moderate
	22	Moderate	Good
	25	Moderate	Moderate
	32	Moderate	Moderate
	35	Moderate	Moderate
	42	Poor	Poor
	50	Good	Good
Euhaline stations	6	High	High
	7	High	High
	11	Moderate	Moderate
	18	Good	Good
	43	Bad	Bad
	44	Good	Moderate
Coastal area	8	Poor	Good
	9	Good	Good
	13	Moderate	Moderate
	15	High	High
	19	High	High
	20	High	High
	23	High	High
	26	High	High
	29	High	High
	33	High	High
	36	High	High
	37	High	High
	41	High	High
	45	Good	Good
	46	High	High
	47	Good	Good
	51	Good	Good

There are different approaches in the definition of reference conditions. Hence, Nielsen et al. (2003) combined the use of historical data and modelling, in Denmark. In the UK, the EUNIS habitat classification is used in developing specific biotope complex reference conditions (Prior et al., 2004). This approach is close to that used in this contribution, in which the association of benthic communities to saline stretches allows the determination of reference values of 'High' and 'Bad biological status', based upon historical data and expert judgement (Borja et al., 2004b). The selection of reference conditions, within the WFD, is a dynamic process: in this, all the member states (MS) should propose and select their own reference conditions for each of the typologies. However, by changing reference conditions the final result can be very different. In this contribution, the reference values are based upon structural parameter values, representative of the corresponding benthic communities associated to each of the typologies and habitats within these typologies. These conditions and results can be revised and modified, during the intercalibration process, in which the other MS can provide further data for validation of these reference values (see Borja et al., 2006c).

Conversely, seasonal changes in benthic communities could affect the results obtained, when comparing with reference values. However, in the reference values selection, seasonal variability was taken into account, as data are derived from samplings that were carried out in different seasons (Borja et al., 2004c). Moreover, it should be taken into account that WFD looks for human-induced changes in the EcoQS, assuming natural variability in the methodology used and the reference conditions. Hence, the use of an index such as AMBI, within the WFD, reduces this problem; this is because it has been demonstrated that this index is very stable throughout the year (in absence of anthropogenic impacts) and is not subjected to seasonality (Salas et al., 2004; Reiss and Kröncke, 2005).

As well as their central role in marine ecosystem functioning, the benthic invertebrates are a well-established 'target' in evaluations of environmental quality status. Various studies have demonstrated that the macrobenthos responds relatively rapidly to anthropogenic and natural stress (Pearson and Rosenberg, 1978; Dauer, 1993). Hence, the use of different univariate and multivariate methodologies, metrics and indices, in measuring such response, has increased dramatically over recent years (see, for a useful summary of biotic indices, Diaz et al., 2004). Many authors (e.g. Washington, 1984) accept that a biotic index is unlikely to be universally applicable, as organisms are not equally sensitive to all types of anthropogenic disturbance; as such, they are likely to respond differently to different types of perturbation. Similarly, they may provide a way to establish a multimetric bioassessment method that, in turn, can be modified for different geographical regions (Borja et al., 2004b,d). Several indices have been proposed for use in marine waters, some of which attempt to include the five-step environmental model of the WFD (Borja et al., 2000, 2003a, 2004b; Simboura and Zenetos, 2002; Rosenberg et al., 2004).

Diaz et al. (2004) state that the 'tautological development of new indices appears to be endemic, self-propagating and rarely justified', recommending that investigators place greater emphasis on evaluating the suitability of indices that already exist, prior to the development of new ones. In this way, the use of existing indices, together with multimetric approaches, could be the most promising way in accomplishing the WFD (Borja et al., 2004b). The research undertaken into indicator and sensitive species, together with their responses to different impact sources (see Hiscock et al., 2004), can lead to an improved understanding of ecosystem functioning, with regard to the assessment of ecological status.

In the case of the typologies determined in the Basque Country, density and biomass are not suitable in determining the biological status, based upon benthic communities. This limitation is because they show a bimodal (non-linear) distribution, in relation to a source of disturbance, with peaks of abundance and biomass both in disturbed and undisturbed locations, making difficult its application in the global benthic health assessment (Pearson and Rosenberg, 1978; Weisberg et al., 1997). The selection of Shannon's diversity, richness and AMBI, into a FA multivariate approach (we propose to call this method as 'Multivariate AMBI' or 'M-AMBI'), appears to be a suitable method in assessing status, which is similar to those used in the US (Engle et al., 1994; Weisberg et al., 1997; Engle and Summers, 1999; Dauer et al., 2000; Llansó et al., 2002b) and UK (Prior et al., 2004).

Conversely, the use of multivariate analysis, such as FA and DA, in assessing benthic quality, has increased in recent years (Gibson et al., 2000; Paul et al., 2001; Smith et al., 2001). These analyses permit the reduction of a large number of variables into a few new parameters explaining most of the variability of the system; this makes it more understandable for stakeholders, in environmental assessment and management (Bald et al., 2005).

In relation to the DA, 87% of the sampling stations, on average, were classified correctly by the discriminant functions (Table 7). Taking into account that WFD establishes that water bodies should be at least in 'Good' status, by 2015, there would be two type of misclassifications with practical consequences: (i) stations at least in 'Good' status that are classified in 'Moderate' status at the most (referred to as a Type A misclassification, in Table 7); and (ii) stations which do not achieve the WFD requirements (in 'Bad', 'Poor' or 'Moderate' status), but are classified at least in 'Good' status by the DA (referred to as a Type B misclassification, in Table 7). In this study, 3% of the locations were misclassified as Type A and 4% of the locations were misclassified as Type B (Table 7).

Table 7
Percentage of cases classified correctly by the DA, percentage of Type A misclassification (sampling stations classified by DA as non-accomplishing with the WFD, when in fact they do) and percentage of Type B misclassification (sampling stations classified by DA as accomplishing with the WFD when in fact the do not) for each of the stretches and in average

Stretches	Correctly classified	Type A	Type B
Oligo- and mesohaline	95.00	0.00	5.00
Polyhaline	91.95	0.00	5.75
Euhaline (estuarine)	92.31	2.56	0.00
Euhaline (coastal)	67.64	9.39	5.83
Average	86.72	2.99	4.14

When studying benthic communities, the selected tools do not always work in the 'expected' way. Hence, there are some concerns in the use of diversity (Heip and Engels, 1974; Zaret, 1982) and AMBI. In the case of AMBI, Simboura (2004), Marín-Guirao et al. (2005), Muxika et al. (2005), and Gómez-Gesteira and Dauvin (2005), have detected some inconsistencies, when using it in isolation i.e. not significant correlations between AMBI and some environmental parameters related to pollution. This is the reason why Borja et al. (2003a, 2004b) and Muxika et al. (2005) advise on the use of the AMBI together with other structural parameters (as in the M-AMBI we propose here). A universal index that works in all systems is unrealistic, because benthic communities are complex and geographically diverse (Engle and Summers, 1999).

In this contribution, some of the stations (3 and 42) show the same increasing trend in benthic and physico-chemical EQR, from 'Bad' or 'Poor', to 'Moderate' or 'Good EcoQS'. Most of these cases are in response to the 'clean-up' of waters, which has produced a continuous increase in bottom water dissolved oxygen and a decrease in the nutrient loads (Franco et al., 2002; Gorostiaga et al., 2004; Borja et al., 2005, 2006b). On the other hand, some other stations (28 and 50) which have also improved their macrobenthic EcoQS, do not show the same trend in the physico-chemical EcoQS. This pattern can be explained because the physico-chemical EcoQS is 'High', throughout the time-series.

Station 28 is located within the Deba estuary, which has been highly polluted due to waste-water inputs from the metallurgical industry (Belzunce et al., 2004). Heavy metals are not taken into account in the physico-chemical EcoQS assessment; this is why physico-chemical EQR was 'High' along all the time series. However, the metallurgical industry incorporated waste-water recovery schemes and the heavy metal load in the waters has decreased since 1996, reaching very low values after 1998–1999 (Belzunce et al., 2004). This evolution can explain the improvement detected in the benthic EQR.

Station 50 is located within the Bidasoa estuary, which supported heavy pressure from urban waste-waters until 1999, when all the discharges were diverted to a submarine outfall outside the estuary (Borja et al., 2005). Such diversion can explain the improvement in the benthic EQR after 1999.

Conversely, some other stations (2, 35 and 44) show decreasing trends in the benthic EQR, from 'High' or 'Good' to 'Moderate' or 'Bad EcoQS'. In these particular cases, the stress on the biological communities can be explained in terms of an excess of nutrients, heavy metals, hydrocarbons and some organic compounds associated with waste-water (both industrial and urban), etc. (Balls, 1992; Windom, 1992; Bock et al., 1999; Lee and Arega, 1999; White et al., 2004). Such a decrease in ecological quality may affect even public health (Herut et al., 1999; Cave et al., 2003; Belzunce et al., 2004).

However, physico-chemical variables, such as dissolved oxygen, nutrients and turbidity are used in the WFD only as supporting elements of the biological elements (Borja et al., 2004b; Bald et al., 2005). Hence, in some cases, benthic communities can show different EcoQS pattern, than the physico-chemical water status. This difference is because most of the factors affecting benthic health can impact through the sediment quality (i.e. metals, organic compounds, etc.); hence, it is necessary to integrate this element into the benthic assessment (Engle et al., 1994; Weisberg et al., 1997; Dauer et al., 2000; Borja et al., 2004e; Borja and Heinrich, 2005). Moreover, the pressures impacting over benthic communities can be not only produced by chemical discharges, but also by biological impacts (i.e. fisheries), morphological alterations (i.e. dredging), etc. (Borja et al., 2006a), even in the presence of 'High physico-chemical EcoQs'.

Finally, FA and DA methodology requires a sufficient amount of data for EcoQS assessment; this can reduce its applicability within those countries where long-time data series are not available. However, taking into account that, by 2006, all the MS will have their own monitoring networks, they should have enough sampling stations to apply this methodology, in the future. Moreover, this methodology could be applied to the new European Marine Strategy Directive (Borja, 2006), which has the same approach than the WFD, in assessing the ecological status of continental shelf and oceanic water bodies.

5. Conclusions

This contribution uses different metrics (species abundance, Shannon Wiener diversity and AMBI), which fulfil the WFD requirements, in assessing EcoQS; multivariate analyses (such as FA and DA) being an objective tool (named here M-AMBI) in such an assessment. Although the results obtained lie close to those obtained by expert judgement, this approach should be intercalibrated with other proposed methodologies, within the European member states, in order to obtain an accurate application.

The classification of water bodies, typologies and the definition of reference conditions are key elements for a successful assessment of the EcoQS. Once these elements have been well established, an appropriate set of metrics is essential for the success of the assessment. This contribution demonstrates the usefulness of both the classification of water bodies into smaller water bodies based upon saline stretches, together with the selection of species richness, the Shannon Wiener diversity index and the AMBI, in determining the benthic status, according to the WFD (and, probably, for the European Marine Strategy).

The EcoQS have improved during recent years along the Basque coast and estuaries, due to the development of many sewerage schemes. In general, the coastal area has achieved a 'Good EcoQS', whereas some estuaries probably would not achieve the 'Good EcoQS' status for 2015, as required in the WFD.

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