ELSEVIER

Journal of Experimental Marine Biology and Ecology 330 (2006) 368 – 382

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

The derivation, performance and role of univariate and multivariate indicators of benthic change: Case studies at differing spatial scales

Victor Quintino [a,*], Michael Elliott [b], Ana Maria Rodrigues [a]

[a] *Universidade de Aveiro, Departamento de Biologia, CESAM-Centro de Estudos do Ambiente e Mar, 3810-193 Aveiro, Portugal*
[b] *Institute of Estuarine and Coastal Studies, University of Hull, Hull, HU6 7RX, UK*

Received 26 July 2005; received in revised form 25 November 2005; accepted 15 December 2005

Abstract

In Europe there is an extensive history of the derivation and use of benthic indicators which parallels similar developments in North America and elsewhere. Most recently, this has increased because major European Union Directives require that indicators of marine benthic change are used to confirm good ecological status quality (as in the Water Framework Directive) and favourable conservation status (as in the Habitats and Species Directive). Furthermore, these indicators have to fit within the current philosophy of the Ecosystem Approach requiring the development and use of Ecological Quality Objectives and Standards. Despite this, comparisons of families of indicators derived by differing methods have not been carried out such that the robust nature of the indicators on differing spatial scales and under differing benthic conditions has not been rigorously assessed. Using case studies from the Portuguese coasts and estuaries, this paper compares and contrasts univariate and multivariate macrobenthic indicators to quantify comparisons of change. The studies indicate the relative value of those indicators at contrasting spatial scales, e.g. in the transition from small areas around coastal submarine outfalls, to the local and regional estuarine and coastal scale. The analysis indicates the difficulties of deriving and using qualitative and quantitative indicators from benthic communities in stable, and in moderately and highly variable environmental conditions in estuarine, coastal and open sea habitats. In some areas, the variability in the indicators and indices within a station and site is as large as that between stations and sites. For those areas studied, there is an adequate quality and quantity of benthic data available for making management decisions but this is unlikely to be the case for all areas. Similarly, the interrogation of the methods shows that while their use and interpretation rely on a good understanding of the biology of the individual species and their responses to physical and polluting stress, that understanding is not yet available for many of the species. Most notably, while the indices and integrative indicators are becoming increasingly sophisticated, many are still dependent on the Pearson–Rosenberg model for organic enrichment hence they require to be validated for physical disturbance and for chemical pollution. Because of these features, the outcome of the analysis has repercussions for the management of coastal and estuarine areas. Although the present study indicates the value of indicators of benthic change for making management decisions at the various scales, further validation is required especially, for example, where one indicator over-estimates the ecological status for poor areas and underestimates it for good areas.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Benthos; Coastal shelf; Estuaries; Indicators; Indices; Organic enrichment; Water framework directive

* Corresponding author. Tel.: +351 234370769; fax: +351 234426408.
E-mail address: vquintino@bio.ua.pt (V. Quintino).

0022-0981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jembe.2005.12.040

1. Introduction

The detection of change in marine and estuarine ecosystems against accepted baselines is a fundamental tenet of estuarine and marine management. Once a change has been detected then management responses are required to address the cause of the change. Many estuarine and coastal management initiatives worldwide, e.g. in North America, Europe and Australia, are required to derive and use environmental quality indices, e.g. the implementation of the European Water Framework Directive (European Commission, 2000) and the US Clean Water Act (Gibson et al., 2000; USEPA, 2002). Those indices are then required to test for departure from a reference or control situation. Thus, there is an increasing need to link indicators to monitoring sensu stricto, i.e. in which change is judged against an a priori derived standard/threshold/reference condition/critical value (McLusky and Elliott, 2004). The results from the use of those indicators then have to be incorporated into management decisions (Fig. 1).

Macrobenthic marine invertebrates have a fundamental role on sediment processes, predator–prey relationships and as bioengineers, and usually have well-defined responses to environmental change, especially those stressors which influence the sediment structure, its chemistry and quality. Because of this, macrobenthic studies have achieved a fundamental role in estuarine and marine impact assessment and marine management (e.g. McLusky and Elliott, 2004). Given the inherent ability of the benthos to integrate sediment quality, many environmental indicators and indices are based on marine macrobenthos. Consequently a large number of techniques have developed to show the degree and nature of the environmental change (Warwick and Clarke, 1991; Elliott, 1994). There is also the advantage that there is a good conceptual understanding of temporal and spatial benthic population and community dynamics in relation to certain types of environmental disturbances, especially organic enrichment (e.g., Pearson and Rosenberg, 1978; Rhoads and Germano, 1986). These, in turn, have produced a large suite of numerical methods which describe environmental change and allow deviation from normal conditions to be quantified (e.g., Elliott, 1994; Borja et al., 2000; Rosenberg et al., 2004). These techniques include graphical, univariate and multivariate statistics and other numerical methods for presenting, describing and interpreting change. However, there is the continued need to question the underlying basis of these paradigms, and to determine the performance and sensitivity of those indices and other numerical models. This is especially so if there is an increasing uncritical use of the indices against stressors for which the indicators were not originally developed.

Biological indices and indicators have been created which summarise ecological status and ecological quality. This study aims to test and validate a suite of those biological indices and indicators, and thus to determine their sensitivity and behaviour, under differing benthic conditions. In particular, there is the need to indicate and explore the management decisions arrived at as the consequence of the output of the benthic analyses. For example, if one index indicated that an area was more polluted than that shown by another index, this result may have financial repercussions following a demand for greater waste treatment.

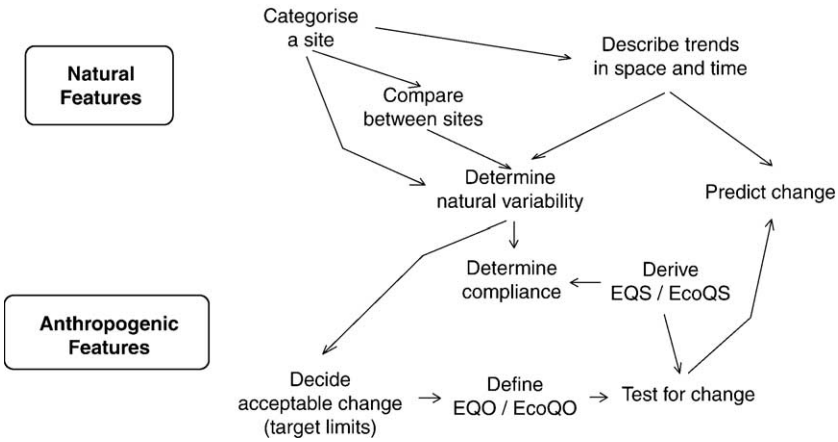

Fig. 1. Schematic representation of the analysis and interpretation of biotic-related information within the establishment and assessment of environmental and ecological quality objectives and standards. EQO — Environmental Quality Objectives; EcoQO — Ecological Quality Objectives; EQS — Environmental Quality Standards; EcoQS — Ecological Quality Standards (from Elliott and Hemingway, 2002).

This paper uses data sets from benthic macrofaunal studies of several sublittoral locations to investigate the following:

- the inherent spatial variability in the indices and indicators (i) among replicates within a site, (ii) among sites, and (iii) among surveys;
- the ability of indices to determine anthropogenic change as shown by the species succession and the community composition;
- the ability to combine indices to produce a more comprehensive description of the benthos and, in contrast;
- the potential for redundancy in the analysis and calculation of indicators of change;
- the differences in the conclusions reached by environmental managers according to different techniques;
- the utility of the indices for implementing management initiatives such as the European Union Water Framework Directive.

2. Methods

Data sets from benthic macrofaunal studies of several sublittoral locations along the western Portuguese coast and the Sado Estuary, central Portugal (Rodrigues and Quintino, 1993; Quintino et al., 1995, 2001; Silva et al., 2004 and unpublished data), were used in the analysis (Fig. 2). The study areas represent several levels of disturbance related to organic enrichment, the severity of which was determined according to established benthic descriptors (Pearson and Rosenberg, 1978): (1) a mixed sewage and pulp mill outfall located on the shelf off Aveiro which does not show organic enrichment effects (Fig. 2, site A); (2) a sewage outfall off Lisbon which corresponds to relatively mild organic enrichment (Fig. 2, site B); (3) a sewage outfall located in the outer Sado Estuary (Fig. 2, site C1) which displays severe organic enrichment, and (4) a pulp mill outfall in the Sado Estuary (Fig. 2, site C2) which represents the most severe organic enrichment of the four sites studied.

Data were obtained by standard benthic methods: Smith–McIntyre 0.1 m^2 grab samples, sieved through 1 mm mesh, and sorted and identified to the highest possible taxonomic resolution. Data were analysed to produce a variety of indices: total abundance (A), total species richness (S), total biomass (B), d (Margalef index), J' (Pielou evenness index), H' (Shannon–Wiener, $\log_e$), $(1-\lambda')$ (Simpson's Index), AMBI (Applied Marine Biological Index, Borja et al., 2000) and its reciprocal (1/AMBI), EQR (Ecological Quality Ratio, calculated according to the UK MBITT Multimetric Approach, Environment Agency, unpublished), BQI (Biological Quality Ratio, Rosenberg et al., 2004), A/S (abundance : species ratio), B/A (biomass : abundance ratio) (Pearson et al., 1982). The reciprocal of AMBI has been used to ensure comparability with other indices in which a poor quality area has a low value rather than a high value. The concept of Ecological Quality Status was used as defined in the European Union Water Framework Directive (European Commission, 2000) and delimited according the UK MBITT Multimetric Approach (Environment Agency, Peterborough, UK, 2005, unpublished) and Rosenberg et al. (2004). The Rosenberg et al. (2004) and AMBI methods require the use of factors describing the response of each species to organic enrichment — the AMBI grouping and the $ES50_{0.05}$ (a probability of occurrence based on the Hulbert rarefaction curve analysis from samples covering a pollution gradient) values. The values given for these factors by their authors have been used when possible or, failing that, recalculated or extrapolated for the other species. In order to keep such extrapolations to a minimum, the BQI method was always calculated using a simplified species data matrix, containing those species whose abundance represent at least 3% of the abundance at any given site. For comparison purposes, the other indices were also calculated with this simplified data matrix, but also with the whole species data set. The calculation of the Ecological Quality Ratio (EQR), according to the UK MBITT Multimetric Approach, is based on the following expression (Dr. A. Miles, Environment Agency, Peterborough, UK, version 1, still undergoing development, unpublished):

$$\mathrm{EQR} = \frac{\left(\left(2 \times \left(1-\left(\frac{\mathrm{AMBI}}{7}\right)\right)\right) + (1-\lambda')\right)}{3} \times \frac{\left(\left(1-\left(\frac{1}{A}\right)\right) + \left(1-\left(\frac{1}{S}\right)\right)\right)}{2}$$

This expression has been derived in order to incorporate the main community parameters required for inclusion under the EU Water Framework Directive (European Commission, 2000). The EQR in the WFD ranges from 0 to 1 and should not be confused with the Ecological Quality Ratio presented by Borja et al. (2004). The suggested tentative EQR thresholds for the purpose of the Ecological Quality Status classification of a site are: "high status": 0.80–1.00; "good status": 0.65–0.79; "moderate status": 0.43–0.64; "poor status": 0.20–0.42; "bad status": 0.00–0.19 (Environment Agency, Peterborough, UK, 2005, unpublished).

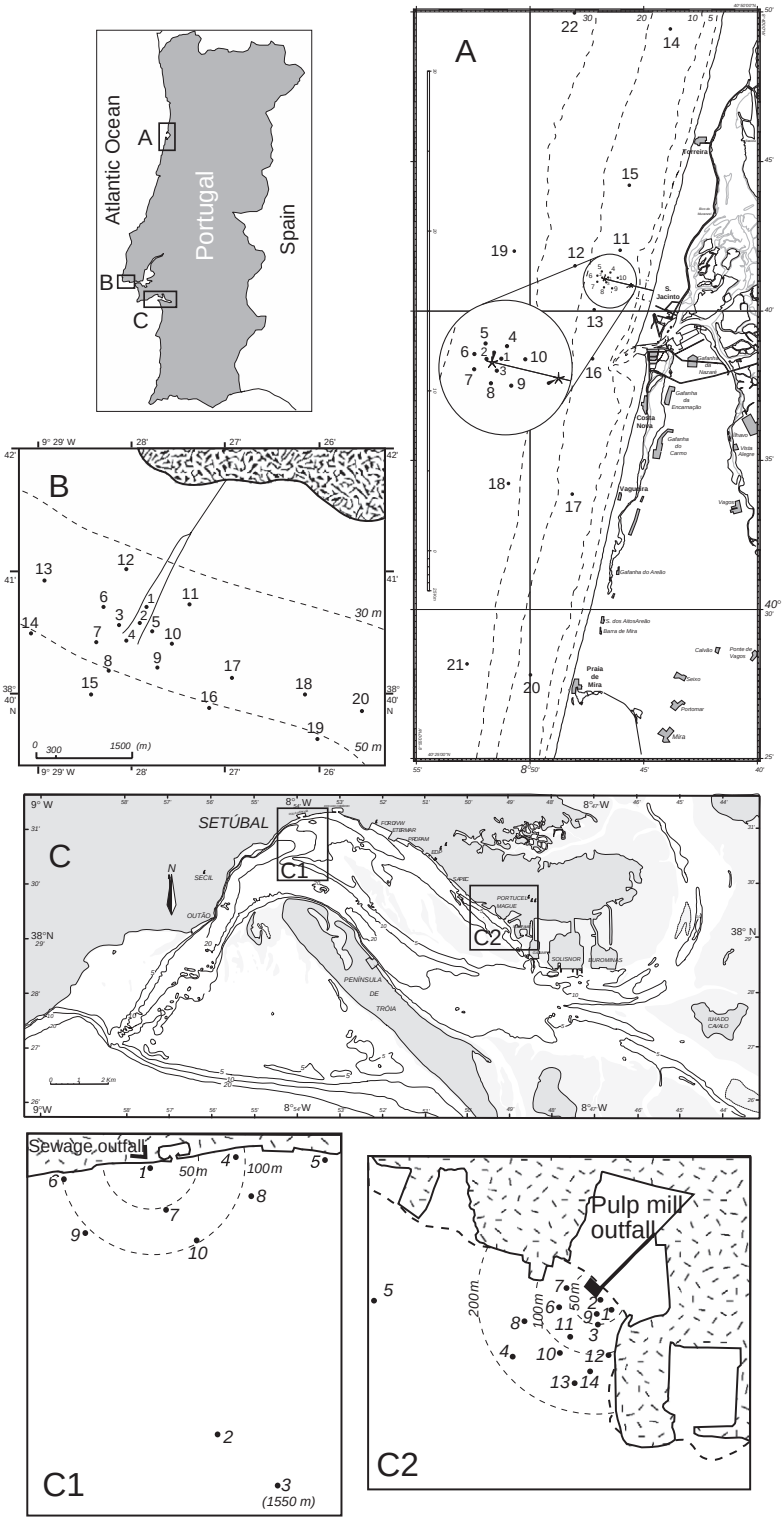

Fig. 2. Location of study areas and sampling sites on the west coast of Portugal. Site A — Mixed sewage and pulp mill outfall (single branch, solid line), coastal shelf off Aveiro; Site B — Sewage outfall (double branches, solid line) off the Tagus Estuary, Lisbon; Site C — Sado Estuary, showing the location of the sewage outfall (C1), and the pulp mill outfall (C2). Intertidal flats in grey. In C1 and C2, the circles (dashed lines) indicate distance from the discharge point. All depth contours in meters.

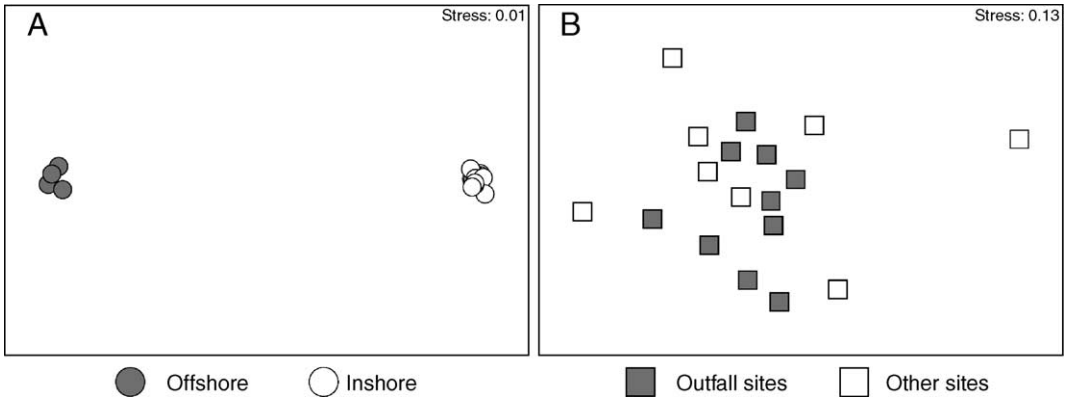

Fig. 3. Ordination diagrams (NMDS) for the simplified species data matrix showing the distribution of the sampling sites from the inshore and the offshore benthic community (A) and, within the inshore community, the distribution of the sampling sites according to their location in the vicinity of the outfall (B). The "outfall sites" correspond to sites 1 to 10 in Fig. 2A.

After calculating the above suite of indices for each site, the resulting matrix was submitted to classification and ordination analyses, using the Spearman rank correlation coefficient to determine the relationships between the indices. Classification analysis used the unweighted pair group mean average algorithm (UPGMA) and ordination analysis used non-metric multidimensional scaling (NMDS). For comparison or descriptive purposes, the original species data matrices were also analysed with the same multivariate techniques, after using the Bray–Curtis coefficient to obtain a similarity matrix between sampling sites. The multivariate analysis was performed with the software PRIMER, v5 (Clarke and Gorley, 2001). The relationship between species data and the indices was also examined with the BIOENV routine in PRIMER (Clarke and Gorley, 2001), which calculates the Spearman rank correlation coefficient between the Euclidean distance matrix between sites, when using the indices values as descriptors, and the Bray–Curtis similarity matrix between sites, when using the species abundances as descriptors.

3. Results

3.1. Exposed coastal shelf area

The benthic communities from the western coastal shelf area under study, located off Aveiro (Fig. 2A), were described in Freitas et al. (2003) and include an inshore fine/very fine sand community, extending up to 25 m depth and an offshore gravel community, occupying the deeper parts of the study area. These two communities are characterized by having distinct species and distinct mean species richness and mean abundance per unit sampling area (Freitas et al., 2003). From July 2000 onwards, a mixed urban sewage and pulp mill effluent have been discharged into this coastal area at approximately 17 m depth (i.e., into the fine/very fine sand habitat). The effects of the operation of this outfall upon the benthic communities were monitored at 22 sampling sites, representing both the inshore and the offshore communities; for the inshore community, sites 1 to 10 were located close to the outfall (Fig. 2A).

The whole data matrix for this site included 151 species whereas a simplified data matrix, containing those species which represented at least 3% of the total abundance of any given sampling site, included 36 taxa. The simplified species abundance matrix, however, retains the most important part of the relevant information from both the inshore and the offshore benthic communities. This is shown by a Spearman rank correlation of 0.98 between the sampling sites' Bray–Curtis similarity matrices obtained with the whole data and with the simplified data, and the clear separation of the two communities by ordination analysis (Fig. 3A). The various quality indices were calculated using the simplified species data matrix. This was done because the reduced data matrix well-represents the whole data matrix but also for practical reasons in that the entire data set was not sufficient to produce reliable $ES50_{0.05}$ values required for the calculation of the BQI index. Also, $ES50_{0.05}$ values given by Rosenberg et al. (2004) omitted species which are very common in southern Europe.

This study area does not show organic enrichment effects; for example, sampling sites located closer to the outfall do not include opportunistic organic enrichment taxa such as *Capitella* spp., *Malacoceros fuliginosa*,

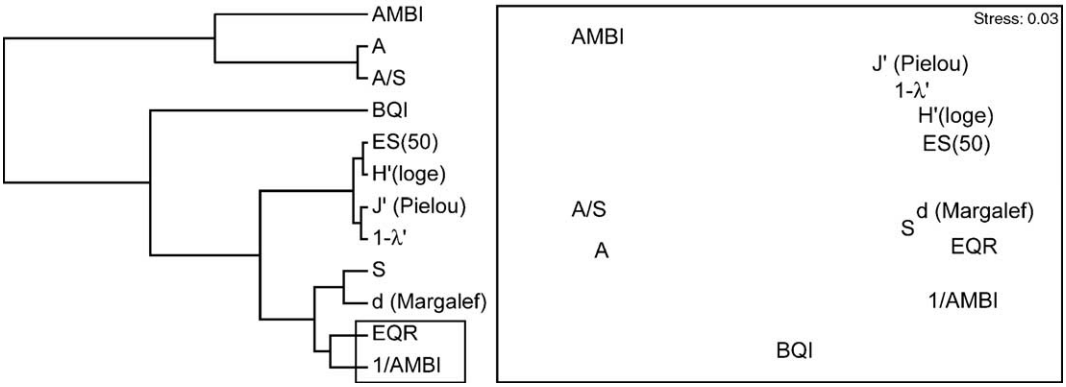

Fig. 4. Classification and ordination analysis of the Spearman correlation matrix between indices in the coastal shelf study area located off Aveiro. Indices were calculated using a simplified species abundance matrix (3% abundance cut-level).

Nassarius reticulatus. The species composition and abundance of the benthic data from samples taken closer to the outfall are similar to those from the remainder of the inshore sampling sites (see the ordination diagram in Fig. 3B).

The classification diagram (Fig. 4) shows that EQR is clearly influenced by 1/AMBI (Spearman rho = 0.853 between the two indices). With this data set, the BIOENV routine (Table 1) showed that the individual indices which best related to species abundance data were some of the primary and derived community variables (A and A/S) and diversity related variables (ES50 and Margalef's d) (Table 1). It is apparent that EQR relates best to the species abundance data matrix, whereas the BQI correlation is close to zero (Table 1). EQR and BQI both identified the lack of organic enrichment effects on the benthic community as shown by the quality status identified by the two indices (Table 2). For example, EQR separated the inshore and the offshore communities, and classified all sites located in the offshore gravel community as "Good" status and all the sites located in the inshore fine/very fine sand community, where the outfall was installed, as "High" status (except site 15, Table 2). Contrary to EQR, BQI only classified one site as "High" status.

Table 1
BIOENV routine for the data set from the coastal shelf area off Aveiro, indicating the Spearman rank correlation (rho) between each index and the simplified species abundances data matrix

rho	Variables
0.810	2
0.768	11
0.730	5
0.721	3
0.671	9
0.668	6
0.611	7
0.563	4
0.528	1
0.383	8
0.279	12
0.044	10

Variables: 1 = S; 2 = A; 3 = d (Margalef); 4 = J' (Pielou); 5 = ES(50); 6 = $H'(\log_e)$; 7 = $1-\lambda'$ (Simpson); 8 = AMBI; 9 = EQR; 10 = BQI; 11 = A/S; 12 = 1/AMBI. Best result: rho = 0.816, with a combination of the variables 2, 3, 5, 7 and 11.

Table 2
Ecological Quality Ratio (EQR) and Biological Quality Index (BQI) values and habitat quality statements (Eco Status and BQI Status) obtained with the simplified species abundance data matrix (3%) in the coastal shelf off Aveiro

Sites	EQR	Eco Status	BQI Status	BQI
1	0.859	High	Good	14.225
2	0.873	High	Good	14.514
3	0.852	High	Good	14.388
4	0.830	High	Good	13.474
5	0.865	High	Good	13.976
6	0.861	High	Good	13.475
7	0.814	High	Good	14.205
8	0.870	High	Good	14.908
9	0.855	High	Good	14.679
10	0.834	High	Good	13.888
11	0.856	High	Good	14.548
12	0.844	High	Good	15.038
13	0.869	High	Good	15.693
14	0.870	High	Good	15.094
15	0.786	Good	Good	13.238
16	0.858	High	Good	15.412
17	0.858	High	Good	14.738
18	0.756	Good	Good	14.421
19	0.778	Good	Good	14.531
20	0.834	High	Good	14.607
21	0.761	Good	High	16.222
22	0.788	Good	Good	13.952

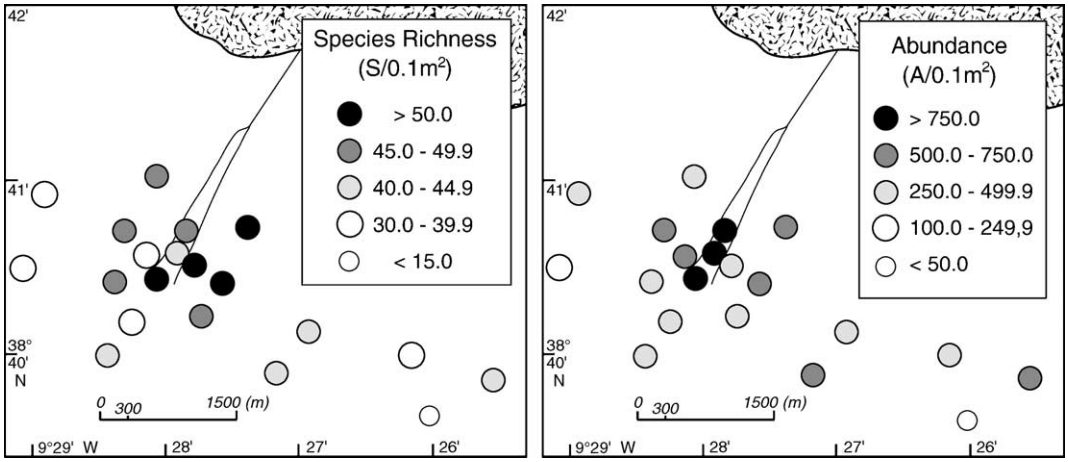

Fig. 5. Distribution of the number of species (S) and the number of individuals (A) per unit sample in the Guia marine outfall coastal shelf area, off Lisbon, Portugal. The solid line represents the two branches of the sewage outfall.

3.2. Sheltered coastal shelf–outer estuarine area

Benthic communities inhabiting the coastal shelf receiving urban effluents from the Guia marine outfall have been previously described (Quintino et al., 2001; Silva et al., 2004). This outfall is the largest marine sewage discharge system in Portugal and was commissioned in 1994. The sampling sites (Fig. 2B) are located between 30 and 60 m depth, mainly on fine/very fine sand with low silt and clay content. This sediment fraction becomes higher with increasing depth and as distance from the estuary diminishes. The benthic dataset used in this study was obtained in 1998 and at that time, organic enrichment effects were mild but noticeable up to 150 m from the outfall diffusers, corresponding to sites 1 to 5 (Fig. 2B). The succession of species number and abundance with increasing distance from the outfall followed the organic enrichment model proposed by Pearson and Rosenberg (1978), and is illustrated in Fig. 5. The high numerical abundance values in the near field were due to the opportunist polychaetes *Capitella* spp. These were absent from the study area before the operation of the outfall but by the time of sampling they had replaced the dominant species and occupied rank 1 or rank 2 in the

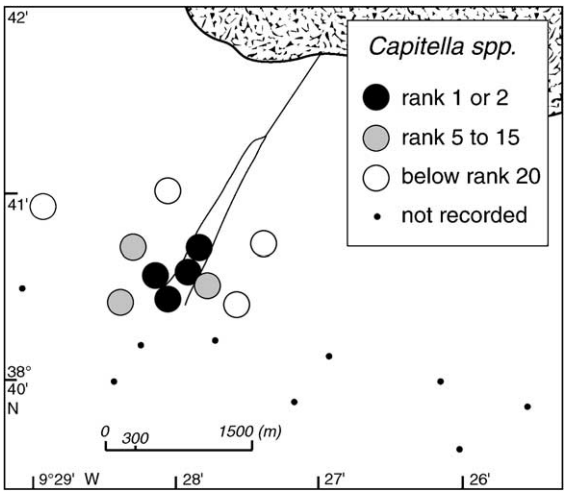

Fig. 6. Distribution of the relative abundance of the organic enrichment opportunist species *Capitella* spp. in the Guia marine outfall coastal shelf area, off Lisbon, Portugal. The solid line represents the two branches of the sewage outfall.

Table 3
BIOENV routine for the data set from Guia outfall, off Lisbon, indicating the Spearman rank correlation (rho) between each index and the simplified species abundances data matrix

rho	Variables
0.498	4
0.476	6
0.468	8
0.428	5
0.409	9
0.409	11
0.404	2
0.388	12
0.372	10
0.365	1
0.345	14
0.286	7
0.281	3
0.193	13

Variables: 1 = S; 2 = A; 3 = d (Margalef); 4 = J' (Pielou); 5 = ES(50); 6 = $H'(\log_e)$; 7 = AMBI; 8 = $1-\lambda'$ (Simpson); 9 = EQR; 10 = BQI; 11 = A/S; 12 = B/A; 13 = W (ABC curves); 14 = 1/AMBI. Best result: rho = 0.740, with a combination of the variables 1, 12 and 14.

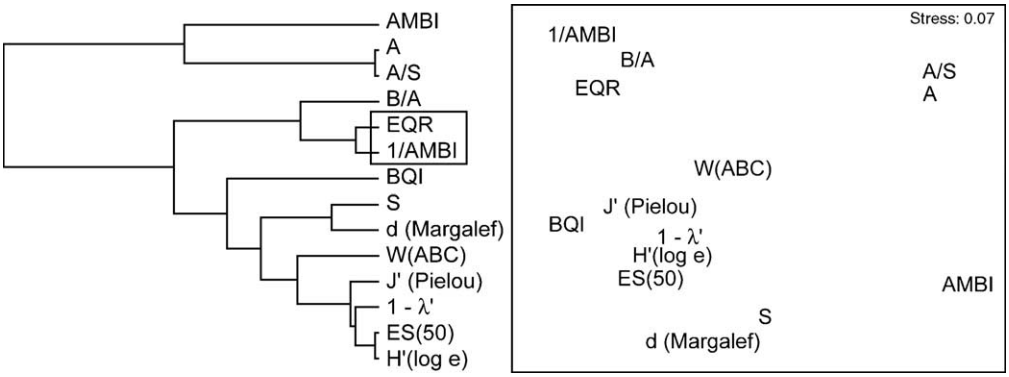

Fig. 7. Classification and ordination analysis of the Spearman correlation matrix between indices in Guia marine outfall study area, off Lisbon. The indices were calculated over a simplified species abundance matrix containing the species above the 3% abundance cut-level.

abundance distribution per species in the near field benthic community (Fig. 6). Despite this, there were no signs of benthic impoverishment associated with excessive organic enrichment in the study area. Table 3 presents the relationships between each of the individual quality indices and the simplified species abundance data matrix. The highest correlations were obtained with variables related to species diversity. The quality status index showing the highest correlation with the species data set was EQR (Table 3). This index again appears closely related to 1/AMBI with Spearman correlation coefficients between the two indices of 0.920 and 0.956, using the simplified (3%) and the full species data matrix, respectively (Fig. 7).

The habitat quality designations based on EQR and BQI are presented in Table 4. Both indices classified site 1 as "Poor" and site 3 as "Moderate" and most of the remaining sites in "Good" status. EQR also classified site 2 as "Moderate" and some of the reference sites, located away from the outfall, in "High" status. BQI failed to make this discrimination but was able to classify the impoverished site 19 (Fig. 5) as "Moderate". In some of the reference sites, the habitat quality designations based on EQR changed from "Good" to "High" when the whole species data were included in the analysis (sites 15 to 18 and 20, Table 4). Although EQR responded mainly to 1/AMBI, the inclusion of the

Table 4
Ecological quality ratio and biological quality index quality statements (Eco Status and BQI Status) obtained with the simplified species abundance data matrix (3%) and the full data set (Eco Status only), in Guia outfall coastal area, off Lisbon

Sites	Eco Status (total spp.)	Eco Status (3%)	BQI Status (3%)
1	Poor	Poor	Poor
2	Moderate	Moderate	Good
3	Good	Moderate	Moderate
4	Good	Good	Good
5	Good	Good	Good
6	Good	Good	Good
7	Good	Good	Good
8	Good	Good	Good
9	High	Good	High
10	High	Good	Good
11	Good	Good	Good
12	High	Good	Good
13	Good	Good	Good
14	Good	Good	Good
15	High	Good	Good
16	High	Good	Good
17	High	High	Good
18	High	Good	Good
19	Good	Good	Moderate
20	High	High	Good

Table 5
Ecological quality ratio and biological quality Index habitat quality statements (Eco Status and BQI Status) obtained with the simplified species abundance data matrix (3%) from the individual replicate samples taken at the near field sites in Guia outfall coastal area, off Lisbon

Site replicates	Eco Status (3%)	BQI Status (3%)
1.1	Moderate	Moderate
1.2	Good	Good
1.3	Poor	Poor
2.1	Good	Good
2.2	Moderate	Good
2.3	Moderate	Good
3.1	Moderate	Moderate
3.2	Good	Moderate
3.3	Moderate	Moderate
4.1	Good	Good
4.2	Good	Good
4.3	Good	High
5.1	Good	High
5.2	Good	High
5.3	Good	High

Fig. 8. Ordination analysis of the Bray–Curtis similarity matrix between near field replicates, sites 1 to 5, in Guia marine outfall, off Lisbon, and representation of the Ecological Quality Ratio (EQR) and Biological Quality Index (BQI) habitat quality statements (Eco Status and BQI Status) on top of the species ordination.

rarer species was thus important to differentiate the whole set of reference sites.

When using the individual replicate samples taken at each site, the indices showed as high a variability within sites as between sites. This is illustrated in Table 5, for the data set from the near field sites only (sites 1 to 5, Fig. 2B). The high species replicate variability which was detected by both EQR and BQI at site 1 was a direct consequence of the inherent variability in the species data from the 3 replicates taken at site 1 and should not be considered an artefact. In addition, as shown on the NMDS plot (Fig. 8), BQI represented better the species gradient than EQR. Among the habitat quality indices, BQI also gave the highest Spearman rank correlation coefficient with species data (Table 6). As with the composite sample, variables related to diversity were still more highly correlated with species data (Tables 3 and 6).

3.3. Estuarine area

The sublittoral benthic communities in the Sado Estuary have been previously described by Rodrigues and Quintino (1993) and Quintino et al. (1995). In comparison to the above case studies, benthic

Table 6
BIOENV routine for the data set from the near field replicates, sites 1 to 5, in Guia marine outfall, off Lisbon indicating the Spearman rank correlation (rho) between each index and the simplified species abundance data matrix

rho	Variables
0.490	6
0.460	5
0.456	7
0.430	13
0.425	1
0.421	4
0.420	3
0.407	12
0.402	8
0.400	2
0.293	14
0.284	11
0.110	10
0.102	9

Variables: 1 = S; 2 = A; 3 = d (Margalef); 4 = J' (Pielou); 5 = ES(50); 6 = $H'(\log_e)$; 7 = $1-\lambda'$(Simpson); 8 = A/S; 9 = B/A; 10 = W (ABC curves); 11 = AMBI; 12 = EQR; 13 = BQI; 14 = 1/AMBI. Best result: rho = 0.664, with a combination of the variables 1, 2, 6, 8 and 14.

Table 7
Number of species (S) and abundance (A) on the benthic community, showing the abundance of *Capitella*spp., in the study areas C1 and C2, located in the Sado Estuary

Sites	Urban sewage outfall (area C1)				Pulp mill outfall (area C2)			
	S/ 0.1 m^2	A/ 0.1 m^2	*Capitella* spp. A/0.1 m^2	*Capitella* spp. Rank	S/ 0.1 m^2	A/ 0.1 m^2	*Capitella* spp. A/0.1 m^2	*Capitella* spp. Rank
1	16	281	255	1	2	2	1	1
2	119	953	–	–	4	202	135	1
3	101	672	–	–	7	9	2	1
4	29	252	124	1	55	376	–	–
5	27	459	379	1	53	610	–	–
6	68	970	9	22	17	78	–	–
7	32	109	5	6	8	29	3	5
8	57	498	–	–	29	475	–	–
9	40	205	–	–	10	15	3	1
10	69	918	–	–	15	77	–	–
11	–	–	–	–	21	162	–	–
12	–	–	–	–	6	28	22	1
13	–	–	–	–	23	75	–	–
14	–	–	–	–	24	87	–	–

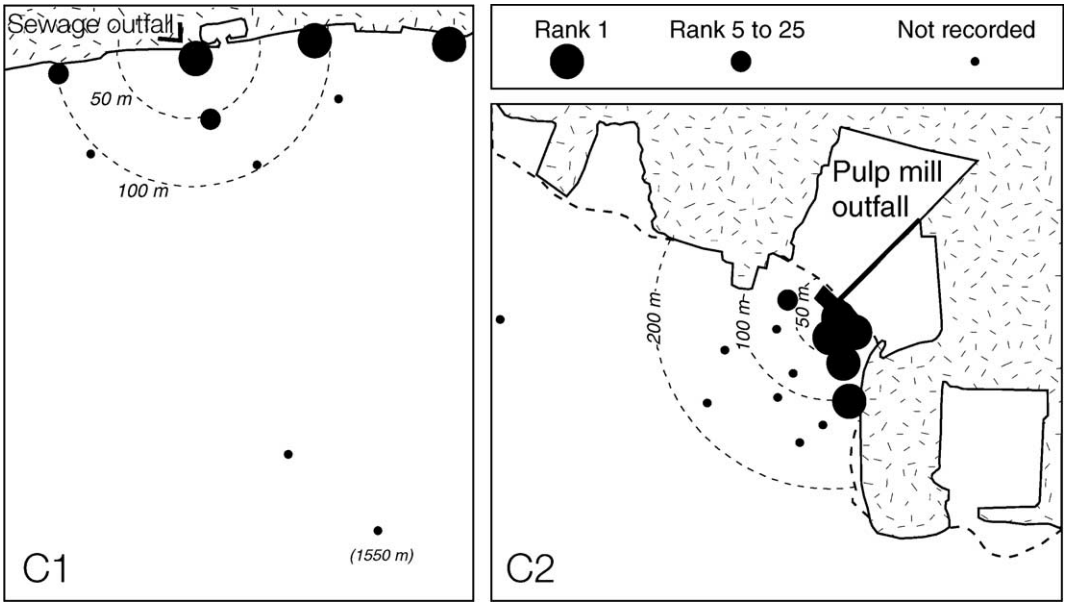

Fig. 9. Relative abundance of *Capitella* spp. in the study areas C1 and C2 located in the Sado Estuary, Portugal. Circles (dashed line) indicate distance from the discharge point.

communities in the study areas C1 and C2 (Fig. 2) are more highly affected by organic enrichment (Table 7, Fig. 9). Adjacent to the urban sewage outfall (C1), sites 1, 4 and 5 are the most affected, with lower species richness and a high dominance by *Capitella* spp. In the pulp mill outfall study area (C2), the most affected sites are also located near to the outfall and upstream of it, closer to the margin, sites 1, 2, 3, 9 and 12 (Fig. 2, Table 7) in which *Capitella* spp. dominated the benthic community. In this area, some of the sites were almost completely defaunated (Table 7).

Table 8
BIOENV routine for the data set from the Sado Estuary sewage outfall (area C1) and pulp mill outfall (area C2) indicating the Spearman rank correlation (rho) between each index and the simplified species abundance data matrix

Urban sewage outfall (area C1)		Pulp mill outfall (area C2)	
rho	Variables	rho	Variables
0.759	4	0.688	10
0.728	10	0.615	8
0.705	7	0.588	5
0.657	6	0.585	1
0.652	8	0.578	9
0.638	9	0.574	12
0.583	5	0.490	6
0.574	12	0.478	3
0.472	1	0.368	7
0.424	3	0.322	4
0.255	2	0.276	11
0.021	11	0.156	2

Variables: 1 = S; 2 = A; 3 = d (Margalef); 4 = J' (Pielou); 5 = ES(50); 6 = $H'(\log_e)$; 7 = $1-\lambda'$ (Simpson); 8 = AMBI; 9 = EQR; 10 = BQI; 11 = A/S; 12 = 1/AMBI. Best result for area C1: rho = 0.867, with a combination of the variables 2, 7 and 10. Best result for area C2: rho = 0.755, with a combination of the variables 1, 4, 5, 8 and 10.

Table 8 presents the rank correlation coefficients between each index and the simplified species abundance data matrix. At the sewage outfall area, the habitat quality indices appeared among the best correlated to species data; BQI occupying the second position, AMBI the fifth and EQR the sixth. At the pulp mull outfall area, BQI and AMBI were the individual indices most highly related to species data and EQR occupied the fifth position. As in the previous case studies, EQR and 1/AMBI were closely related (Fig. 10). For the sewage outfall area, Spearman correlation coefficients between the two indices were 0.951 and 0.988 using the simplified (3%) and the full species data matrix, respectively. For the pulp mill outfall area, the values were, respectively, 0.965 and 0.916. Table 9 presents the EQR and BQI values and associated quality statements for the sewage outfall area, using the simplified species data matrix. Table 10 presents the EQR values and habitat quality descriptors based on simplified and full species data matrices. As shown in Table 9, almost no differences were seen between the quality statements. Both identified sites 1, 4 and 5 in the poorest habitat quality status, although the BQI tended to classify the most disturbed sites as a

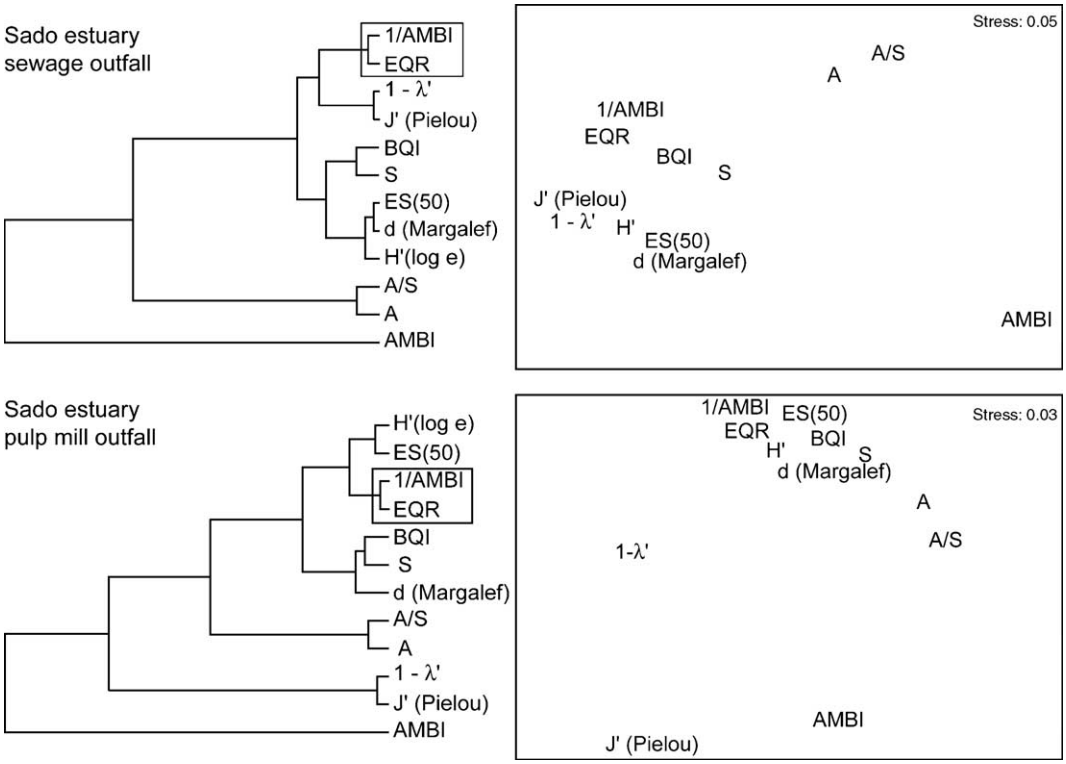

Fig. 10. Classification and ordination analysis of the Spearman correlation matrix between indices for the Sado Estuary sewage outfall and pulp mill outfall. The indices were calculated using a simplified species abundance matrix containing the species above the 3% abundance cut-level.

worse ecological status than the EQR. The good agreement between both indices' quality statements and the species data is shown in Fig. 11. As shown in Table 10, almost no change is noticed in the final EQR classification when reducing the number of active species to the 3% abundance cut-level.

Table 11 presents the EQR and BQI values and associated habitat quality statements for the pulp mill outfall area, using the simplified species data matrix. In this case the EQR failed to classify as "Bad" the most disturbed sites, namely, those showing greatly degraded benthic communities. BQI also represented species gradients more accurately than EQR (Fig. 12).

Table 9
Ecological Quality Ratio (EQR) and Biological Quality Index (BQI) values and habitat quality statements (Eco Status and BQI Status) obtained with the simplified species abundance data matrix (3%), in the Sado Estuary sewage discharge area, C1, off Setúbal, Portugal

Sites	EQR	Eco Status	BQI Status	BQI
1	0.142	Bad	Bad	1.950
2	0.675	Good	Good	12.972
3	0.675	Good	Good	13.214
4	0.409	Poor	Poor	4.567
5	0.206	Poor	Bad	2.702
6	0.745	Good	Good	13.688
7	0.740	Good	Good	12.509
8	0.771	Good	Good	13.589
9	0.691	Good	Good	11.974
10	0.738	Good	Good	12.290

4. Discussion

The present study has shown that in many areas, the variability in the environmental indicators and indices within a station and/or site can be as large as that between stations and sites, thus reflecting the inherent spatial heterogeneity in benthic systems (Elliott and O'Reilly, 1991). However, that natural heterogeneity must be borne in mind when making quality assessments of an area and, for example, when such quality assessments may result in demands for installing expensive effluent treatment systems. The indices used here, which represent a subset of the major number of methods for analysing the benthos (Ferraro et al., 1989, 1991; Elliott, 1994; Diaz et al., 2004; Bortone, 2005) are unlikely to discriminate between subtle changes in community structure although are sufficient to detect large scale differences which can then be attributed to a well-defined stressor (Rees et al., 1990; MAFF, 1993).

Some of the indices used here rely on the need for a good understanding of the biology of the individual species and their responses to physical and anthropogenic stresses. This is implicit, for example, in the AMBI group assigned to each species (Borja et al., 2000) which may have to be assumed for con-generic species where precise information does not exist for a given species. Whereas there is a good indication of the tolerance of species to organic enrichment (e.g., Rosenberg et al., 2004) to derive their $ES50_{0.05}$ values for some areas, that understanding is not yet available for many other species, especially within other biogeographic areas such as those studied here. While the $ES50_{0.05}$ values mentioned in Rosenberg et al. (2004) were used here, they require validation and further deriving for all areas.

As shown here, the indices and integrative indicators such as the AMBI, BQI and MBITT, are becoming increasingly sophisticated and used in many different geographical areas. However, they are still dependent on the Pearson–Rosenberg model for organic enrichment; hence they must be validated for other stressors such as physical disturbance and chemical pollution. This is especially necessary where the WFD requires the determination of changes to ecological quality status as the result of physical modifications in waters classified as Heavily Modified Water Bodies (European Commission, 2000). Opportunist species responding to organic enrichment, such as *Capitella* and *Malacoceros*, will not be the same as species responding to physical disturbance, such as *Chaetozone* and *Polydora* (pers. obs.) hence factors derived for one stressor should be re-derived for other stressors.

The community responses described here merely reflect changes in community structure in which there is an assumed cause and effect relationship related to a single or multiple stressor. In the examples used here, that main stressor was organic enrichment and the patterns were investigated according to the Pearson–Rosenberg paradigm and the suite of acknowledged changes to benthic community structure following organic inputs (McManus and Pauly, 1990; Elliott, 1994). However, these inshore and estuarine areas will also be subject to other stressors and so indices dependent only or mainly on the organic enrichment paradigm will be insufficient. Therefore, cause and effect, or "burden of evidence" relationships need to be further elucidated, namely requiring linked bioassays such as those within the sediment quality triad (Chapman et al., 1987). As such, the roles and responses of the benthic community indices and indicators should be further determined and interpreted with regard to other bioassessment approaches, namely ecotoxicological responses.

Table 10
Ecological Quality Ratio habitat quality statements (Eco Status) obtained with the full data set and the simplified species abundance data matrix, in the Sado Estuary sewage discharge area, C1, off Setúbal, Portugal

Sites	Full data set (total species)			Simplified data set (3% cut-level)		
	$S/0.1$ m^2	$A/0.1$ m^2	Eco Status	$S/0.1$ m^2	$A/0.1$ m^2	Eco Status
1	16	281	Bad	11	274	Bad
2	117	951	Good	22	695	Good
3	99	666	Good	25	497	Good
4	27	240	Moderate	14	206	Poor
5	26	458	Poor	13	431	Poor
6	66	955	Good	22	739	Good
7	30	104	Good	16	88	Good
8	55	494	Good	21	386	Good
9	40	205	Good	16	153	Good
10	66	902	Good	17	709	Good

The use of indicators is becoming an integral part of decision support systems for coastal zone management (e.g. Gibson et al., 2000; Whitfield and Elliott, 2002; Bortone, 2005) and there is a plethora of techniques for determining change at all levels of biological organisation (e.g. Bayne et al., 1988; McLusky and Elliott, 2004). However, as shown here, there is the need for further testing and validation of the indicators. Although the present study has indicated the value of indicators of benthic change for making management decisions at the local and regional scale, further validation is required over these and wider scales, such as within and between biogeographic regions. It is especially important to indicate the way environmental managers would use the results and conclusions of the use of one or more indices. For example, in some of the cases studied here EQR appears to over-estimate the ecological status for "poor" areas and underestimate it for "good" areas in comparison to the BQI. Hence, if there are different but non-consistent responses of the different indices, these will lead to doubt in managers' minds regarding the value of the methods. It is of particular note that the AMBI did not indicate the almost abiotic nature of a heavily polluted area. More importantly, they can produce confusion regarding whether remediation measures are needed.

The present study also shows the importance of data manipulation techniques for complex community data. The matrix reduction procedure here was aimed at assessing data redundancy and preventing the possibility of spurious relationships caused by the derivation and use of the methods. Whereas this reduction affected

Fig. 11. Ordination analysis of the Bray–Curtis similarity matrix between sites in Sado Estuary sewage outfall (area C1) and representation of the Ecological Quality Ratio (EQR) and Biological Quality Index (BQI) habitat quality statements (Eco Status and BQI Status) on top of the species ordination.

the final habitat quality statements for the Guia sewage outfall, this was not the case for the Sado estuary sewage outfall. This is relevant to environmental managers who may receive quality assessments made by scientific appraisal although there may be financial repercussions of misclassifying an area. If one index indicates that an area was degraded then an industry or discharger will be liable for restoring the area to better ecological status. However, if a second index indicates that the area was not in such a degraded condition there would be no such financial repercussion. This is particularly relevant for implementing the EU WFD, if a particular area is found to be of moderate or poor ecological status then remediation, usually at high expense, will be required to achieve a better ecological status (European Commission, 2000). If that conclusion is erroneous then there will be needless expenses incurred in remediation. Within the WFD, the critical boundary is between Good and Moderate Ecological Status such that areas classified as the latter, or worse, will require to be remedied.

Table 11
Ecological Quality Ratio (EQR) and Biological Quality Index (BQI) values and habitat quality statements (Eco Status and BQI Status) obtained with the simplified species abundance data matrix (3%), in the Sado Estuary pulp mill discharge area, C2

Sites	EQR	Eco Status	BQI Status	BQI
1	0.214	Poor	Bad	1.074
2	0.231	Poor	Bad	1.560
3	0.514	Moderate	Poor	4.511
4	0.611	Moderate	High	14.817
5	0.594	Moderate	Good	14.319
6	0.634	Moderate	Moderate	7.684
7	0.620	Moderate	Poor	7.005
8	0.464	Moderate	Moderate	8.314
9	0.634	Moderate	Moderate	7.706
10	0.594	Moderate	Good	11.055
11	0.664	Good	Moderate	9.319
12	0.256	Poor	Bad	2.221
13	0.668	Good	Moderate	10.273
14	0.690	Good	Good	11.124

A further area of caution is the adequacy of the data for generating the indices and for making management decisions (see also Bortone, 2005). While the benthic data for the areas studied here were adequate for making management decisions, it is unlikely to be the case for all areas. Within the implementation of the WFD, all areas need to be assessed for departure from "Good Ecological Status" irrespective of whether data are available. However, it is likely that areas with perceived problems will have been studied and so data are available. It is also likely that the environment protection agencies, following a risk-based approach, will give less attention to areas lacking in perceived or recorded problems.

The analysis here has indicated several other problems which need to be addressed before implementation of environmental management initiatives such as the WFD. These include:

- scores to be calculated for all European areas;
- data truncation by removal of rare species needs to be further validated; those rare species removed would be the higher ranked $ES50_{0.05}$ (lower ranked AMBI) scores and thus this makes degraded areas appear worse;
- the behaviour of indices based on sample replicates as opposed to summed or single replicate values needs testing further as the patterns may be smoothed and

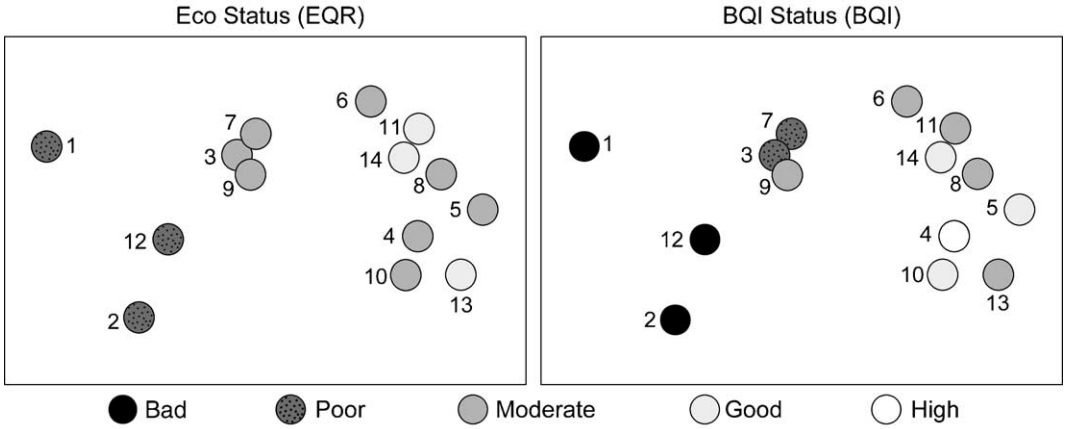

Fig. 12. Ordination analysis of the Bray–Curtis similarity matrix between sites in Sado Estuary pulp mill outfall (area C2) and representation of the Ecological Quality Ratio (EQR) and Biological Quality Index (BQI) habitat quality statements (Eco Status and BQI Status) on top of the species ordination.

problem areas may be underestimated by combining replicates;

- the need to further test assumptions inherent in the methods; the present use of the methods includes several assumptions (e.g. individual species responses) which may reduce the discriminatory behaviour of the methods;
- there is the need to determine the response to hydromorphological change through sediment/depth changes rather than just as a response to pollution/organic enrichment changes;
- there is the need to further test the weighting of the indices and the way in which they can be combined into integrative indicators such as EQR (i.e. what is the value of adding in other indices than AMBI into EQR).

5. Conclusions

The present study has shown that the variability in the indicators and indices within a station and site may be as large as that between stations and sites. For the areas studied, there is an adequate quality and quantity of benthic data available for making management decisions but this is unlikely to be the case for all areas for which indicators are required. Similarly, although the methods rely on a good understanding of the biology of the individual species and their responses to physical and chemical polluting stress, that understanding is not yet available for many of the species. As shown here, the indices and integrative indicators are becoming increasingly sophisticated but many are still dependent on the Pearson–Rosenberg model for organic enrichment hence they require to be validated for physical disturbance and for non-organic and ecotoxicological responses following chemical pollution. Although the present study has indicated the value of indicators of benthic change for making management decisions at the local, regional, national and European scale, further validation is required, e.g. the EQR appears to over-estimate the ecological status for poor areas and underestimate it for good areas. These different but non-consistent responses of the different indices, for example AMBI did not indicate the almost abiotic nature of a heavily polluted area, will therefore lead to doubt in managers' minds regarding the value of the methods. Most importantly, the outcome of the use of the indicators has a financial dimension such that areas misclassified as being "poor status" will then require expensive remediation measures.

Acknowledgements

The authors wish to thank the UK Marine Benthic Invertebrate Task Team (Environment Agency, Peterborough, UK) under the direction of Dr Alison Miles for valuable discussions regarding the indices derived by the MBITT. Also, our thanks to Professors Dan Dauer and Rutger Rosenberg and Dr Angel Borja for valuable discussion on the analyses. The authors are grateful for the comments provided by Dr Bob Whitlatch and the anonymous reviewers. **[SS]**

References

Bayne, B.L., Clarke, K.R., Gray, J.S. (Eds.), 1988. Biological Effects of Pollutants: Results of a Practical WorkshopMar. Ecol. Prog. Ser., vol. 49, pp. 1–278.

Borja, Á., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft bottom benthos within European estuarine and coastal environments. Mar. Pollut. Bull. 40, 1100–1114.

Borja, Á., Franco, J., Valencia, V., Bald, J., Muxika, I., Belzunce, M.J., Solaun, O., 2004. Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. Mar. Pollut. Bull. 48, 209–218.

Bortone, K. (Ed.), 2005. Estuarine Indicators. CRC Press, Boca Raton, Florida.

Chapman, P.M., Dexter, R.N., Long, E.R., 1987. Synoptic measures of sediment contamination, toxicity and infaunal community composition (the sediment quality triad) in San Francisco Bay. Mar. Ecol. Prog. Ser. 37, 75–96.

Clarke, K.R., Gorley, R.N., 2001. PRIMER v5: User Manual/Tutorial. PRIMER-E, Plymouth, UK. 91 pp.

Diaz, R.J., Solan, M., Valenta, R.M., 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. J. Environ. Manag. 73, 165–181.

Elliott, M., 1994. The analysis of macrobiological data. Mar. Pollut. Bull. 28 (2), 62–64.

Elliott, M., Hemingway, K.L. (Eds.), 2002. Fishes in Estuaries. Blackwells, Oxford. 636 pp.

Elliott, M., O'Reilly, M.G., 1991. The variability and prediction of marine benthic community parameters. In: Elliott, M., Ducrotoy, J.-P. (Eds.), Estuaries and Coasts: Spatial and Temporal Intercomparisons. Olsen and Olsen, Fredensborg, Denmark, pp. 231–238.

European Commission, 2000. Water Framework Directive: Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the community action in the field of water policy. Official Journal of European Communities.

Ferraro, S.P., Cole, F.A., DeBen, W.A., Swartz, R.C., 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, Washington, USA. Can. J. Fish. Aquat. Sci. 46, 2157–2165.

Ferraro, S.P., Swartz, R.C., Cole, F.A., Schults, D.W., 1991. Temporal changes in the benthos along a pollution gradient: discriminating the effects of natural phenomena from sewage-industrial waste water effluent. Estuar. Coast. Shelf Sci. 33, 383–407.

Freitas, R., Rodrigues, A.M., Quintino, V., 2003. Benthic biotopes remote sensing using acoustics. J. Exp. Mar. Biol. Ecol. 285/286, 339–353.

Gibson, G.R., Bowman, M.L., Gerritsen, J., Snyder, B.D., 2000. Estuaries and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance. EPA 882-B-00-024. U.S. Environmental Protection Agency, Office of Water, Washington, DC. www.epa.gov/owow/monitoring/.

MAFF, 1993. Analysis and interpretation of benthic community data at UK sewage disposal sites. Aquat. Environ. Monit. Rept., vol. 37. MAFF Direct. Fish. Res, Lowestoft, UK.

McLusky, D.S., Elliott, M., 2004. The Estuarine Ecosystem; Ecology, Threats and Management, 3rd edn. Oxford University Press, Oxford. 216 pp.

McManus, J.W., Pauly, D., 1990. Measuring ecological stress: variations on a theme by RM Warwick. Mar. Biol. 106, 305–308.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16, 229–311.

Pearson, T.H., Duncan, G., Nuttal, J., 1982. The Loch Eil project: population fluctuations in the macrobenthos. J. Exp. Mar. Biol. Ecol. 56, 305–321.

Quintino, V., Picado, A.M., Rodrigues, A.M., Mendonça, E., Costa, M.H., Bordalo Costa, M., Linggaard-Jorgensen, P., Pearson, T.H., 1995. Sediment chemistry — infaunal community structure in a southern European estuary related to solid-phase Microtox® toxicity testing. Neth. J. Aquat. Ecol. 29 (3–4), 427–436.

Quintino, V., Rodrigues, A.M., Ré, A., Pestana, M.P., Silva, S., Castro, H., 2001. Sediment alterations in response to marine outfall operation off Lisbon, Portugal: a sediment quality triad study. J. Coast. Res. Special Issue 34, 535–549.

Rees, H.L., Moore, D.C., Pearson, T.H., Elliott, M., Service, M., Pomfret, J., Johnson, D., 1990. Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites. Scot. Fish. Info. Pamphlet, vol. 18. DAFS, Aberdeen.

Rhoads, D.C., Germano, J.D., 1986. Interpreting long-term changes in benthic community structure: a new protocol. Hydrobiologia 142, 291–308.

Rodrigues, A.M., Quintino, V., 1993. Horizontal biosedimentary gradients across the Sado estuary, Western coast of Portugal. Neth. J. Aquat. Ecol. 27 (2–4), 449–464.

Rosenberg, R., Blomqvist, M., Nilsson, H.C., Cederwall, H., Dimming, A., 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Mar. Pollut. Bull. 49, 728–739.

Silva, S., Ré, A., Pestana, P., Rodrigues, A.M., Quintino, V., 2004. Sediment disturbance off the Tagus estuary, western Portugal: chronic contamination, sewage outfall and runoff events. Mar. Pollut. Bull. 49, 154–162.

USEPA, 2002. Federal Water Pollution Control Act (as amended through P.L. 107–303, November 27, 2002). (33, U.S.C. 1251 et seq.), 230 pp. URL: http://www.epa.gov/region5/water/cwa.htm.

Warwick, R.M., Clarke, K.R., 1991. A comparison of methods for analysing changes in benthic community structure. J. Mar. Biol. Assoc. UK 71, 225–244.

Whitfield, A.K., Elliott, M., 2002. Fishes as indicators of environmental and ecological changes within estuaries — a review of progress and some suggestions for the future. J. Fish Biol. 61, 229–250 (Suppl. A).