Hydrobiologia (2007) 587:101–112
DOI 10.1007/s10750-007-0697-3

ECSA39

Applicability of ecological evaluation tools in estuarine ecosystems: the case of the lower Mondego estuary (Portugal)

H. Teixeira · F. Salas · M. A. Pardal · J. C. Marques

Abstract In accordance with the Water Framework Directive guidelines (WFD, 2000, European Communities Official Journal L327 2000/60/EC), classification schemes and ecological evaluation tools (based on benthic invertebrate fauna data sets from 1990 to 2002) were applied in the lower Mondego estuary. Two distinct scenarios could be tested due to the implementation of mitigation practices in 1999, following a long eutrophication process, which started by the early 1980s. Some discrepancies in the results were found by the application of the different indices. The AMBI index (accounting for taxonomic composition) and the ABC method (accounting for abundance and biomass k-dominance patterns) classifications often disagreed with those based on species diversity (Margalef and Shannon-Wiener). The ambiguous results made the classification a complex task to achieve, contrary to the Directive's objective of maintaining it simple and clear. Our results suggest the necessity of adjusting some of the indices and their ranges to estuarine characteristics, namely to account the typical dominance and abundance of some particular species. These aspects are not taken into consideration by some of the indices proposed, which are more adapted to typical marine conditions. Based on our results, these widely applied indices might still improve their efficiency in estuarine systems allowing their use in the resembling types already established within the new Directive agenda.

Keywords Water framework directive · Ecological indicators · Transitional waters · Classification tools and ecological quality status

Introduction

Since the Water Framework Directive (WFD, 2000/60/EC) became effective the approach to water issues has changed significantly. The concept of ecological status developed in this Directive, requires new methods capable of distinguishing different levels of ecological quality for the classification of surface water areas (including transitional and coastal waters).

According to WFD, composition and abundance of benthic invertebrate fauna are included within the biological quality elements for the

Guest editors: M. J. Costa, H. Cabral & J. L. Costa
Towards an integrated knowledge and management of estuarine systems

H. Teixeira (✉) · M. A. Pardal · J. C. Marques
IMAR - Institute of Marine Research, c/o
Department of Zoology, University of Coimbra,
Coimbra 3004-517, Portugal
E-mail: helianateixeira@ci.uc.pt

F. Salas
Department of Ecology and Hydrology, Faculty of
Biology, University of Murcia, Murcia 30100, Spain

definitions of ecological status. Benthic communities are usually considered more adequate than those of the pelagic domain to evaluate the status of an aquatic ecosystem. In fact, due to their limited mobility, benthic organisms are more sensitive to local disturbance, and due to their permanence over seasons, they integrate the recent history of disturbances that might not be detected in the water column (Warwick, 1993; Cardoso et al., 2004).

Nevertheless, experience demonstrates that none of the available measures of disturbance effects may be considered ideal. But it seems that the combination of different measures results in a good toolset for determining the ecological quality status. In this sense, the results of the TICOR project (Typology and Reference Conditions for Portuguese Transitional and Coastal Waters) (Bettencourt et al., 2004) include a method that combines a suite of indices. This work group also suggested that the biomass parameter should be taken into account, since in organic enriched situations it is considered to be an important metric for the effects of extra energy inputs into a system. Following those guidelines, a combination of the Shannon-Wiener index (Shannon & Weaver, 1963), Margalef index (Margalef, 1968), the AMBI Marine Biotic index (Borja et al., 2000) and the ABC curves method by means of the *W*-statistic (Warwick, 1986; Clarke, 1990), was recommended for Portuguese transitional and coastal waters. Simultaneously, a multimetric approach designed by Borja et al. (2003, 2004) is also being adopted in other Member States (Prior et al., 2004). Similarly to the TICOR project, Borja et al. (2003, 2004) considered the possibility of combining different metrics (Shannon-Wiener index, Specific Richness and the Marine Biotic index AMBI) into a general index of ecological quality.

At the moment, these two classification tools cannot be considered of universal application before being used in several different environments, to test their abilities in distinguishing ecological status and to define the correct ecological status classes' boundaries. Note that, according to the WFD, the ecological status classes' boundaries of an index should be set as a function of the reference conditions defined for each water type. Yet, many types still lack their reference condition values.

This paper's purpose is to evaluate the behaviour of these two methodological proposals in an estuarine system. Besides the Portuguese methodology, one of the reasons for choosing Borja et al. methodology as the second approach to be tested is the fact that this one, unlike the Portuguese, indicates how to derive the Ecological Quality Ratio (EQR) the Directive requires.

Studies carried out in the Mondego estuary (Portugal), in the past 15 years, provide a large database and a comprehensive information background (e.g. Marques et al., 1993, 2003; Dolbeth et al., 2003; Cardoso et al., 2004; Pardal et al., 2004) allowing a comparison of the two multimetric approaches and an assessment of the reliability of the final ecological status designations.

Due to the combination of highly variable freshwater discharge and mesotidal regime, this type of estuary is the most representative for transitional waters in Portugal (Type A2), covering about 93% of the total area for transitional waters, increasing the importance of having adequate assessment tools.

In the Mondego estuary two distinct scenarios can be tested due to the implementation of mitigation practices in 1999, following a long eutrophication process. Moreover, the presence of two different channels with different physical and chemical characteristics suffering from distinct environmental impacts provides the ground for further analysis of the methodology response.

Materials and methods

Study site

The Mondego estuary is located on the Atlantic coast of Portugal (40°08′ N, 8°50′ W). The lower reaches of this estuary extend for about 8 km and cover an area of approximately 3,4 km^2, comprising two contrasting arms, northern and southern, separated by an island (Fig. 1). The northern arm is deeper (4–8 m during high tide, tidal range 1–3 m) and constitutes the principal navigation

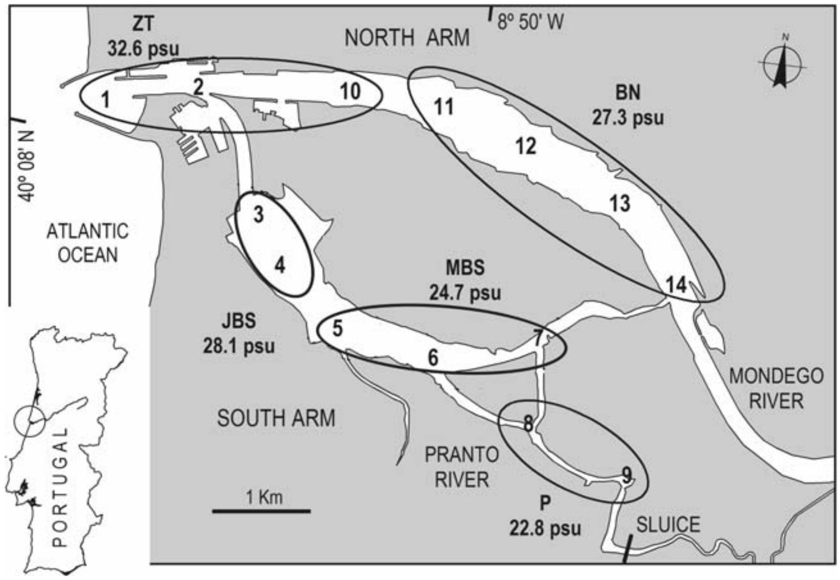

Fig. 1 The Mondego estuarine system. Distribution of the 14 subtidal sampling stations along the north and south arms and the Pranto river. Indication of the 5 distinct zones settled after the predefined stations (ZT, BN, JBS, MBS, P) and the mean salinity values during the study period at each zone

channel and the location of the Figueira da Foz harbour. The southern arm is shallower (2–4 m during high tide, tidal range 1–3 m) and is almost silted up in the upper zones, so the freshwater outflow is mainly via the northern arm. Circulation in the southern arm is mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary. The discharge from this tributary is controlled by a sluice and is regulated according to water needs of rice fields in the Mondego Valley. Harbour facilities and consequent dredging activities, on the north arm, cause physical disturbance of the bottoms, while freshwater discharge from agricultural areas in the river valley results in an excessive nutrient release into the south arm (Marques et al., 2003). These anthropogenic activities coupled with specific physical characteristics and climate conditions have contributed to an increase of environmental stress (Dolbeth et al., 2003; Cardoso et al., 2004; Pardal et al., 2004). This system is recently and gradually recovering from the effects of eutrophication after the mitigation measures implemented in the south arm, which improved transparency of the water and decreased nutrient loading.

Sampling procedures

The subtidal soft-bottom communities were sampled in springtime at 13 stations in the lower Mondego estuary in the years 1990 and 1992, and with an additional one in the downstream area in 1998, 2000 and 2002. The sampling sites, covering the last 8 km of the estuary, are located 1 km apart from each other. In accordance to Rodrigues (2004), this lower part of the estuary can be divided in different zones and each of them includes a group of the sampled stations as shown in Fig. 1. In the northern arm, ZT zone comprises stations with the strongest marine influence, and BN is the zone with the most unstructured sediments due to the effects of dredging activities. In the southern arm the zones differ on the extent of eutrophication symptoms (JBS and MBS, respectively less and more affected) and on the content of organic matter within the sediments, being higher on the upstream stations (P).

At each station six replicates of soft substrate were taken with a Van Veen LGM grab of 496 cm^2. The samples were sieved in situ using a 1 mm mesh sieve bag and fixed in 4% buffered formalin. At each station the following environmental factors were measured: salinity, temperature, pH, dissolved oxygen, silica, chlorophyll *a*, ammonia, nitrites, nitrates and phosphates in water; and a sediment sample was also collected, to quantify organic matter content and determine granulometry. At the laboratory, organisms collected were identified at species level, counted and, from 1998 onwards, their ash-free dry weight (AFDW) was assessed, after combustion for 8 h at 450°C.

Indices application

The biological data were submitted to the following indices suggested for Portuguese transitional waters (Bettencourt et al., 2004): Margalef index (*D*) (Margalef, 1968), *W*-Statistic (*W*) (Warwick, 1986; Clarke, 1990), Shannon-Wiener index (*H′*) (Shannon & Weaver, 1963), and the Marine Biotic index AMBI (BO) (Borja et al., 2000). In the same way, these two last indices (Shannon-Wiener and AMBI) and the Specific Richness (S) were considered in the classification described in Borja et al. (2003, 2004). Formulas adopted for each index are:

$$D = (S - 1)/\text{loge } N$$

$$H' = -\Sigma\, p_i \log_2 p_i$$

$$W = \Sigma\, (B_i - A_i)/50\, (S - 1)$$

$$BC = [(0)(\%GI) + (1,5)(\%GII) + (3)(\%GIII) + (4,5)(\%GIV) + (6)(\%GV)]/100$$

Where: *S*—number of species; *N*—total number of individuals; p_i—proportion of abundance of species *i* in a community where species proportions are p_1, p_2, $p_{3 \ldots} p_n$.; *n*—number of species; B_i—biomass of species *i*; A_i – abundance of species *i*; GI—Ecological Group I (species very sensitive to organic enrichment and present under unpolluted conditions); GII—Ecological Group II (species indifferent to enrichment, always in low densities with non-significant variations with time); GIII—Ecological Group III (species tolerant to excess of organic matter enrichment, these species may occur under normal conditions, but their populations are stimulated by organic enrichment); GIV—Ecological Group IV (second-order opportunist species, mainly small sized polychaetes); GV—Ecological Group V (first-order opportunist species, essentially deposit-feeders).

The diversity indices and *W*-Statistic were applied using the PRIMER 5 software package (Software package from Plymouth Marine Laboratory, UK) (Clarke & Gorley, 2001). The Marine Biotic index was applied using the AMBI© software (Borja et al., 2003; AZTI, 2004).

In TICOR classification, the definition of ecological classes boundaries for each index was based upon theoretical information in Bellan-Santini (1980), Ros & Cardell (1991), Warwick & Clarke (1994), Molvær et al. (1997) and Borja et al. (2000) (Table 1). These initial borders were set until reference conditions are established for Portuguese transitional and coastal water types. Besides that, apart from the valuations given by each index, the combination of three of them (depending on the type of data available) provides a joint valuation that is established as shown in Table 2. Such evaluation does not have to be considered as a rigid tool, and in the cases in which two situations have to be considered (i.e. Moderate/Poor; or Good/Moderate) our knowledge on the system will be a key element to decide to which information given by each of the indices should be given more importance. On the other hand, the method described by Borja et al.

Table 1 Ecological levels according to indices values considered in TICOR methodology

Margalef (*D*)	AMBI (BC)	Diversity (*H′*)	*W*-statistic	Ecological status
>4	0–1.2	>4	0.1–1	High
>4	1.2–3.3	3–4	0.1–1	Good
2.5–4	3.3–4.3	2–3	–0.1–0.1	Moderate
<2.5	4.3–5.5	1–2	<–0.1	Poor
<2.5	5.5–7	<1	<–0.1	Bad

Table 2 Classification of benthic ecological status after the valuation of three indices combination (Bettencourt et al. 2004)

Combination of three of the selected indices (depending on the type of the data available)			Ecological Status
High	High	High	High
High	High	Good	High/Good
High	Good	Good	Good
Good	Good	Good	
Good	Good	Moderate	Good/Moderate
Good	Moderate	Moderate	Moderate
Moderate	Moderate	Moderate	
Moderate	Moderate	Poor	Moderate/Poor
Moderate	Poor	Poor	Poor
Poor	Poor	Poor	
Bad	Poor	Poor	Poor/Bad
Bad	Bad	Poor	Bad
Bad	Bad	Bad	

(2003, 2004) establishes a correspondence between the different index ranges and Equivalent Assigned Values (EAV). These EAVs are used to give the assessment status by summing them and dividing by the number of indices considered in the multimetric method, in this case three. This final multimetric output provides an EQR that determines the ecological status (see details in Table 3). Though the indices ranges in this last methodology might not be adequate to our data (since it was not developed for Portuguese water types), the EQR estimate makes it interesting to compare with the Portuguese approach.

Data treatment

Pearson's correlations were applied to analyse and to identify any significant parallelisms between the patterns of variation of different indices. To test for the independence of the two multimetric methodologies, data of final ecological classifications from each methodology were arranged in a 3×2 contingency table and analysed using a two-tailed Chi-square statistic.

A MDS analysis was performed with the PRIMER 5 (Software package from Plymouth Marine Laboratory, UK) (Clarke & Gorley, 2001). Data (species abundance) were transformed by double square root and a Bray Curtis similarity matrix was calculated. The zones were labelled with the status class derived from each classification to verify how they related according to the ecological status criterion. An ANOSIM analysis was carried out to determine how separate those groups were on a scale of 0 (groups are indistinguishable) to 1 (all similarities within groups are less than any similarities between groups).

All statements of statistics significance were based on $\alpha = 0.05$.

Results

The identification of the sampled material provided a total of 107 species: 32 in 1990, 29 in 1992, 34 in 1998, 50 in 2000 and 61 in 2002. During the entire study period the benthos was dominated by

Table 3 Calculating the Ecological Quality Ratio (EQR) and the correspondent Ecological Status according to Borja et al. (2003, 2004) methodology

Richness (S)	AMBI (BC)	Diversity (H')	EAV	EQR	Ecological status
>60	0–1.2	>4.8	1	0.8–1	High
45–60	1.2–3.3	3.6–4.8	0.75	0.6–0.8	Good
30–45	3.3–4.3	2.4–3.6	0.5	0.4–0.6	Moderate
15–30	4.3–5.5	1.2–2.4	0.25	0.2–0.4	Poor
0–15	5.5–7	0–1.2	0	0–0.2	Bad

species such as the polychaetes *Alkmaria romijni* Horst, 1919, *Streblospio shrubsolii* (Buchanan, 1890) and *Hediste diversicolor* (Müller, 1776), the decapods *Carcinus maenas* (Linnaeus, 1758) and *Crangon crangon* (Linnaeus, 1758), the isopod *Cyathura carinata* (Krøyer, 1847) and the bivalves *Scrobicularia plana* (da Costa, 1778) and *Cerastoderma edule* (Linnaeus, 1758).

The indices estimates obtained at each zone and for each of the sampling periods are shown in Table 4. The AMBI index was not able to detect any variation of the estuary during the whole study. According to this index the Mondego estuary presented always a Good ecological status (values ranging from 1.4 to 3.3) with all zones described as slightly polluted. Ecological group III, characteristic of unbalanced benthic communities, was dominant in almost all samples. Unlike AMBI, the Margalef, Shannon-Wiener and *W*-statistic (applied when biomass data was available) indices were able to detect different ecological situations through time along the five estuarine areas considered.

The significant correlations (Table 5) found between the indices included in TICOR methodology strongly support the notion that the 4 indices always follow the same numerical tendency, but this is not always reflected on the final ecological evaluation. This is clearer in the AMBI that presented always the same final result (Good) not being able to discriminate the variations of the system along years.

Despite the significant correlations, results interpretation shows some inconsistencies between evaluations provided by the different indices. For example, in 1998 the results provided by AMBI for zone P did not agree with those of the other three indices. AMBI pointed this zone as Good (BC = 3.1) while the others considered it Bad or Poor ($W = -0.2$, $D = 0.9$ and $H' = 1.4$). The low number of species present ($n = 8$) and the dominance of small sized polychaetas like *A. romijni* (1278 ind m^{-2}) and *S. shrubsolii* (369 ind m^{-2}) explain the low diversity values and the switch between biomass and number curves of the ABC method, signs of polluted communities.

Table 4 compares both multimetric approaches. The methodology suggested for Portuguese transitional waters tended to assign higher ecological status to an area than Borja's et al. (2003, 2004) method. The contingency table analysis by means of the Chi-square test confirmed that the two multimetric methodologies originated significantly different ecological classifications ($\chi^2 = 12.923$, $P < 0.01$). Nevertheless, prior to the application of mitigation measures, both approaches indicated poorer ecological status throughout the estuary.

The 2-dimensional MDS configuration, based on species abundances, indicated some difficulty (stress 0.16) in displaying the relationships between areas of different ecological evaluation estimated by the two approaches (Fig. 2). Groups of zones with equal ecological status are not easily defined. ANOSIM tests, applied to assess the significance of ecological segregation, indicate that Borja et al. (2003, 2004) methodology failed to distinguish the different zones according to the ecological status criterion ($R = 0.126$, $P = 0.056$). On the other hand, the TICOR approach achieved a significant distinction of the different ecological zones ($R = 0.226$, $P = 0.038$). Even though the low value of the Global R indicates little segregation of the groups, the faunal assemblages of the ecological groups defined by TICOR are significantly different between each ecological status.

Discussion

Our results have shown that multimetric methodologies can be appropriate tools when dealing with classification of coastal systems in the scope of the WFD, 2000. As shown, the ANOSIM test for the TICOR methodology confirmed the existence of distinct groups according to the ecological status criterion indicating that the different ecological classifications produced by this methodology were real and could be reflected by the MDS configuration. Moreover, frailties found on some of the indices responses, discussed further on, point towards the multimetric approach as a more reliable one due to the complementary nature of each index' ecological principles. However, the multimetric nature of a methodology is not warrant of an accurate assessment. As our

Table 4 Indices applied on the distinct zones of the estuary from 1990 to 2002 following TICOR and Borja et al. (2003, 2004) procedures

Year	Zones	Margalef		Richness		Biotic coefficient			Shannon-Wiener			ABC method		TICOR		Borja
		D	Eco level	*S*	EAV	BC	Eco level	EAV	*H'*	Eco level	EAV	*W*	Eco level	Ecological status	EQR	Ecological status
1990	ZT	1.7	Bad/Poor	6.0	0	2.6	Good	0.75	2.0	Poor	0.25			Moderate	0.3	Poor
	BN	2.8	Moderate	15.0	0	2.2	Good	0.75	3.0	Good	0.5			Good	0.4	Moderate
	JBS	3.1	Moderate	17.0	0.25	2.6	Good	0.75	3.3	Good	0.5			Moderate	0.5	Moderate
	MBS	2.6	Moderate	22.0	0.25	3.1	Good	0.75	1.2	Poor	0			Moderate	0.3	Poor
	P	1.6	Bad/Poor	11.0	0	3.3	Good	0.75	2.1	Moderate	0.25			Moderate	0.3	Poor
1992	ZT	1.9	Bad/Poor	11.0	0	2.4	Good	0.75	2.0	Poor	0.25			Moderate	0.3	Poor
	BN	1.9	Bad/Poor	10.0	0	2.8	Good	0.75	2.3	Moderate	0.25			Moderate	0.3	Poor
	JBS	2.9	Moderate	13.0	0	2.1	Good	0.75	3.2	Good	0.5			Good	0.4	Moderate
	MBS	2.3	Bad/Poor	13.0	0	3.1	Good	0.75	2.1	Moderate	0.25			Moderate	0.3	Poor
	P	1.1	Bad/Poor	7.0	0	3.1	Good	0.75	2.1	Moderate	0.25			Moderate	0.3	Poor
1998	ZT	3.5	Moderate	19.0	0.25	1.4	Good	0.75	3.3	Good	0.5	0.3	Good/High	Good	0.5	Moderate
	BN	3.4	Moderate	16.0	0.25	2.2	Good	0.75	3.2	Good	0.5	0.4	Good/High	Good	0.5	Moderate
	JBS	1.1	Bad/Poor	6.0	0	2.8	Good	0.75	1.5	Poor	0.25	–0.2	Bad	Poor	0.3	Poor
	MBS	1.7	Bad/Poor	13.0	0	3.0	Good	0.75	2.1	Moderate	0.25	0.1	Moderate	Moderate	0.3	Poor
	P	0.9	Bad/Poor	8.0	0	3.1	Good	0.75	1.4	Poor	0.25	–0.2	Bad	Poor	0.3	Poor
2000	ZT	5.8	Good/High	38.0	0.5	3.2	Good	0.75	3.1	Good	0.5	0.0	Moderate	Moderate	0.6	Moderate
	BN	2.0	Bad/Poor	9.0	0	2.7	Good	0.75	2.6	Moderate	0.5	0.2	Good/High	Moderate	0.4	Moderate
	JBS	3.4	Moderate	13.0	0	2.4	Good	0.75	3.5	Good	0.5	0.4	Good/High	Good	0.4	Moderate
	MBS	1.6	Bad/Poor	13.0	0	3.0	Good	0.75	2.2	Moderate	0.25	0.1	Moderate	Moderate	0.3	Poor
	P	1.1	Bad/Poor	8.0	0	3.1	Good	0.75	1.6	Poor	0.25	0.1	Good/High	Moderate	0.3	Poor
2002	ZT	5.9	Good/High	33.0	0.5	2.0	Good	0.75	3.8	Good	0.75	0.2	Good/High	Good	0.7	Good
	BN	3.1	Moderate	21.0	0.25	2.9	Good	0.75	2.2	Moderate	0.25	–0.1	Bad	Moderate	0.4	Moderate
	JBS	2.8	Moderate	24.0	0.25	2.7	Good	0.75	1.1	Poor	0	0.0	Moderate	Moderate	0.3	Poor
	MBS	2.9	Moderate	23.0	0.25	3.2	Good	0.75	3.2	Good	0.5	0.1	Good/High	Moderate	0.5	Moderate
	P	2.4	Bad/Poor	14.0	0	2.9	Good	0.75	3.0	Moderate	0.5	0.2	Good/High	Moderate	0.4	Moderate

Table 5 Pearson correlations between the different indices included in TICOR methodology estimated from 1990 to 2002 for the 5 zones previously defined in the Mondego estuary

	Margalef	AMBI	Shannon-Wiener
AMBI	–0.44*		
Shannon-Wiener	0.65**	–0.51**	
W-statistic	0.45	–0.43	0.70**

Values for *W*-statistic are regarding 1998, 2000 and 2002 (*$P \leq 0.05$; **$P \leq 0.01$)

results also show that the two methodologies tested still origin a considerable discrepancy on the final classification results.

As mentioned, the differences between the two approaches are on the specific richness component, the diversity component, the distinct method of defining the joint valuation of the indices, and on the fact that TICOR could also account for biomass, not applicable in Borja's approach. Some of these differences were clearly reflected on the final classification outcome, while others' contribution to the disparity found might be more difficult to account for. For example, it is not easy to quantify the difference added by the inclusion of the *W*-statistic in one of the methodologies, or the difference added by the definition of final ecological status by means of an EQR instead of a combination of each index classification. Nonetheless, there are evidences in the results of the contribution of *W*-statistic index to the final ecological outcome. In some situations low diversity values, of either D or H', pointed to poorer ecological status, but *W*-statistic showed values indicating abundance and biomass distribution patterns typical of non-disturbed communities (e.g. zone P in 2000). In such cases, and considering the frail behaviour of AMBI index, the *W*-statistic result was crucial to determine the final ecological status. *W*-statistic index can be considered of universal applicability, i.e. the interpretation of measurements is independent of geographical area or type of system, since it provides an absolute rather than a comparative measure of pollution-induced disturbance (Warwick & Clarke, 1994; Bettencourt et al., 2004). This proved to be helpful when reference conditions are undefined.

Regarding diversity indices, despite the presence of the Shannon-Wiener index in both multimetric approaches and their similar way of evaluating specific richness, Margalef index versus number of species, each defined different ranges for the 5 ecological levels, resulting on a more demanding valuation of the indices in Borja's et al. methodology. There were 12 situations where species number in Borja et al. gave lower results than Margalef index in TICOR. The same could be observed for the Shannon-Wiener results, where in 17 circumstances Borja et al. approach gave poorer results than TICOR. The

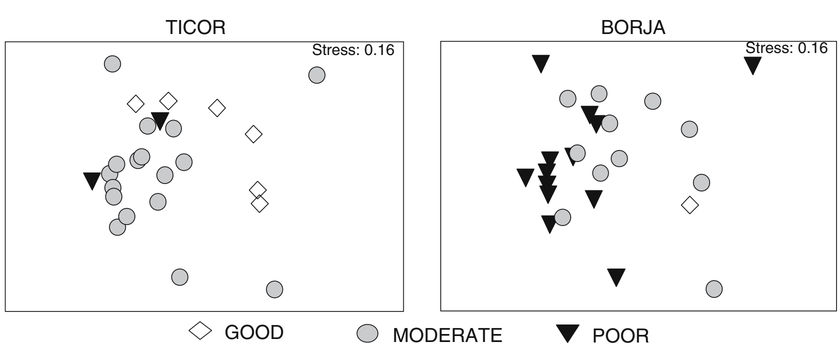

Fig. 2 Two-dimensional MDS plot of taxa abundance data of 25 sampling zones during the study period from 1990 to 2002. Each sampling occasion is labelled according to ecological status established through application of the multimetric methods described in TICOR (Bettencourt et al., 2004) and Borja et al. (2003, 2004)

worse performance of Borja et al. methodology in this study, as revealed by the ANOSIM results, is due to the fact that indices' boundaries were not developed accounting for the studied water type. Reference conditions for this methodology refer to Basque country (northern Spain) coastal water types (Borja et al., 2003). It is known that a lower diversity (regarding specific richness and communities' equitability) should be expected for estuarine systems (Wilson & Elkaïm, 1992) and it should be reflected in less demanded indices' boundaries for the five ecological status classes. The boundaries suggested for Portuguese classification schemes are not yet adjusted to any specific estuarine or coastal type, nevertheless they seem to be more compliant with estuarine conditions than those of the second methodology tested. Anyhow, both multimetric methodologies can be adjusted in function of the type of system studied.

In this study there was also evidence for some vulnerabilities of the indices included in the methodological approaches, hence their results are to be accepted with caution. The main problems associated with diversity indices (i.e. Shannon-Wiener index) refer to the usual dominance of some species non-indicative of pollution, such as *Hydrobia ulvae* (Pennant, 1777) or *S. plana*, which occur naturally at high densities in this estuary (Cardoso et al., 2005; Verdelhos et al., 2005).

The *W*-statistic index also revealed some limitations when in the presence of non-pollution indicators (e.g. *Elminius* sp., *C. edule*) whose dominance (large abundances but representing low biomass) led to the definition of non-polluted conditions as disturbed (e.g. ZT in 2000, BN in 2002, JBS in 2002). The high *C. edule* juveniles' densities observed in 2002 might have occurred due to the climate interference in these bivalves' mortality and recruitment events, as described by Beukema et al. (2001) and Strasser et al. (2003). In 2001, a high mortality was observed among this estuary's species probably due to the significant decrease in salinity and low temperatures as a result of the floods registered in 2001/2002 harsh winter. Misclassifications of unstressed communities as highly stressed due to dense recruitments events had already been observed in other studies (Beukema, 1988; Dauer et al., 1993). Warwick & Clarke (1994) had already alerted for the fact that evidence of pollution or disturbance indicated by the ABC method should be viewed with caution if the species responsible for the ''polluted'' configurations are not polychaetes. The patchy distribution of the small non-pollution indicators *Elminius* sp. influenced *W*-statistic outcome by forcing an abundance/biomass distribution pattern similar to those of disturbed communities.

The presence of the species *Elminius* sp. could also have had some influence on AMBI index results. Recent guidelines for the use of this index (Borja & Muxika, 2005) advice the removal of hard-bottom substrata individuals, since it was developed specifically to soft-bottom communities. In this case, the inclusion of Elminius sp. resulted in 46.9% non-assigned taxa in ZT 2000 sample. A percentage over 20% of non-assigned taxa would produce doubtful results, and over 50% would invalidate AMBI's use (Borja & Muxika, 2005).

As mentioned, the two arms of the Mondego estuary constitute two different subsystems with distinct environmental features. Granulometric structure of the sediments and daily salinity fluctuations are the most important factors conditioning the subtidal macrofauna distribution in this lower region of the estuary, and the cause for the biological differences between both arms (Marques et al., 1993; Rodrigues, 2004). Regular dredging activities intensify this difference, increasing sediments instability in the north arm. The lack of structure in the northern arm sediments and the strongest salinity oscillation leads to a clear macrofaunal impoverishment (the lowest abundances are found in this arm), with many epifaunal species [*H. ulvae*, *C. maenas, C. crangon, Lekanesphaera hookeri* (Leach, 1814)] present along this arm. Infaunal species (*H. diversicolor*, *S. shrubsolii*, *S. plana*) occur mainly in the most upstream stations, less affected by dredging (Marques et al., 1993; Rodrigues, 2004). The zone near the mouth of the estuary (ZT) is characterized by the highest species diversity but also by the lowest abundance of individuals. Due the vicinity of coastal waters, this area registered a great variation in the species type encountered during the study period.

On the other hand, infaunal species [*A. romijni, S. shrubsolii, Polydora ciliata* (Johnston, 1828), *C. carinata*] are dominant in the south arm (Rodrigues, 2004). The structured sediments in this zones and their high organic matter content are responsible for local macrofauna assemblages. In fact, inner areas of this arm, with the highest organic matter values, registered the highest abundances of individuals in the study area. South arm downstream zone, where the eutrophication effects are negligible, still preserves *Zostera noltii* Hornemann, 1832 beds and was considered the most structured area regarding intertidal communities (Cardoso et al., 2004). These meadows had a positive influence over the subtidal communities of JBS zone, which presented often the better ecological quality among south arm zones.

Despite the differences in community structure and the different environmental impacts in the two arms, according to AMBI index, they shared the same status in terms of benthic community health. The dominance of species belonging to ecological group III (Borja et al., 2000) in the two arms of the estuary resulted in identical ecological classification. It is clear in the present work that the robustness of this index is reduced when applied in naturally stressed communities such as estuarine ones. As recognise Wilson & Elkaïm (1992), some areas in estuaries are naturally dominated by opportunistic organisms by virtue of salinity or other stressors, therefore it is sometimes difficult to separate pollution-induced changes from natural variation. This could explain partially why AMBI was not able to distinguish ecological status of the inner areas of the south arm (with symptoms of eutrophication and organically enriched) from downstream areas (non-eutrophied) more affected by daily salinity variations. Muxika et al. (2005) have evaluated this index performance towards several impact sources and also found evidence of the difficulty in detecting sand extraction impacts when these actions are not followed by an increased abundance of opportunistic species. In our system, where extractions take place twice a year, there was never detected a substitution of Ecological Group III tolerant species by Ecological Group IV or Ecological Group V opportunistic species. The classification of species as indicators of different degrees of pollution, which constitutes the base of AMBI, often contains subjective elements; in fact, the interpretation regarding the meaning of the presence of a given species may be ambiguous (Warwick, 1993). To improve its performance in transitional systems there are still two problems to be solved on this AMBI index: (a) regarding Ecological Status classes boundaries, that need to be more discriminating and adjusted to estuarine characteristics; (b) and regarding the assignment of species' ecological group.

Indices' weaknesses discussed here suggest that the assessment of the ecological quality of a certain ecosystem should rely on approaches based on more than one index since their combination makes up for the defects of each one.

In this paper some of the issues regarding multimetric methodologies, for ecological assessment in the scope of WFD, namely in Portugal, were raised. Besides indices ecological classes boundaries adaptations, other aspect regarding AMBI index should be explored, namely on the assignment of species' Ecological Group. *A. romijni*, considered presently belonging to EG III, should probably be included in EG IV since there are evidences in this estuary that it behaves ecologically similarly to *Capitella capitata* (Fabricius, 1780) (unpublished results). Further investigation and proposals regarding these matters should be made in this direction. Yet, this paper's data are insufficient to allow reliable proposals or adjustments to calibrate these methodologies for Portuguese estuarine water types. New series of data is being gathered, and eventually more data on the qualitative evolution observed in this estuary since 1999 until recently will contribute to this tools' adjustment.

Nevertheless some problems are still to be solved in the WFD scope. For instance:

(a) Shall we standard the mesh sieve to be used (500 μm or 1 mm)? It is known that small species connected to organic enrichment are usually not retained by 1 mm mesh sieve and due to that biased results may arrive from different methodologies (Schlacher & Wooldridge, 1996; Thompson et al., 2003). Besides, more than detect an impact, the Directive's objective is to express the structural and functional quality of the ecosystems. Such a characterization demands a more exhaustive evaluation of the biological communities; therefore a 500 μm mesh sieve is needed. There has also been argued that, for estuarine environments, 500 μm mesh sieve should be adopted (NEAGIG, 2004);

(b) Besides system type, should the existing different habitats also be accounted for when establishing reference conditions? The existing literature (e.g. Boström et al., 2002; Cardoso et al., 2004) gives good indications that inside the same system, benthic communities may change drastically depending on the existence of macrophyte beds. Salinity and sediments structure should also be regarded for reference conditions purposes (Ysebaert et al., 2003). Within an estuary, species assemblages will differ depending on the zones considered (McLusky, 1990). From the head of the estuary towards its mouth, abiotic factors such as salinity and sediment characteristics (grain size, mud content) gradually change, conditioning species distribution. These environmental variables are determinant of the type of community that will colonize an area, influencing parameters such as diversity, species richness, composition and abundance of individuals (McLusky, 1990). These features are habitat specific and they are basic to correctly assess benthic community health within WFD scope, hence to define the Ecological Classes. As pointed by Diaz et al. (2004), habitat quality assessment remains somewhat tenuous without habitat classification system. Moreover, to identify representative, distinct, or at risk habitats at appropriate scales consistent with conservation priorities, common operational procedures are necessary to facilitate both the small and large scale characterization of habitat on scales ranging from cm to km (Zacharias et al., 1999; Diaz et al., 2004). Therefore this issue should be adressed within the WFD.

Though these issues are well investigated and documented, they lack reference within WFD implementation guidelines. The Intercalibration and Monitoring processes going on in all Member States will in time try to answer to these questions, managing to define the boundaries between classes and the methods to estimate sound EQR values.

Acknowledgements The present study was carried out in the scope of the research project Typology and Reference Conditions for Transitional and Coastal Portuguese Waters—TICOR, funded by INAG—Instituto da Água (Portugal), and Dynamic model of stress induced changes on benthic communities—DYNAMOD (POCTI/MGS/37431/2001), funded by FCT (Portuguese National Board of Scientific Research).

References

AZTI, 2004. AZTI tecnalia: investigación marina y alimentaria. http://www.azti.es.

Bellan-Santini, D., 1980. Relationship between populations of amphipods and pollution. Marine Pollution Bulletin 11: 224–227.

Bettencourt, A., S. B. Bricker, J. G. Ferreira, A. Franco, J. C. Marques, J. J. Melo, A. Nobre, L. Ramos, C. S. Reis, F. Salas, M. C. Silva, T. Simas & W. Wolff, 2004. Typology and Reference Conditions for Portuguese Transitional and Coastal Waters. Instituto da Água. Instituto do Mar, Lisbon.

Beukema, J. J., 1988. An evaluation of the ABC-method (abundance/biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden Sea. Marine Biology 99: 425–433.

Beukema, J. J., R. Dekker, K. Essink & H. Michaelis, 2001. Synchronized reproductive success of the main bivalve species in the Wadden Sea: causes and consequences. Marine Ecology Progress Series 211: 143–155.

Borja, A., J. Franco & I. Muxika, 2003. Classification Tools for Marine Ecological Quality Assessment: the Usefulness of Macrobenthic Communities in an Area Affected by a Submarine Outfall. ICES C. M. 2003/J:02,. ICES, Copenhagen.

Borja, A., J. Franco & V. Pérez, 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin 40: 1100–1114.

Borja, A., J. Franco, V. Valencia, J. Bald, I. Muxika, M. J. Belzunce & O. Solaun, 2004. Implementation of the European water framework directive from the Basque country (Northern Spain): a methodological approach. Marine Pollution Bulletin 48: 209–218.

Borja, A. & I. Muxika, 2005. Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. Marine Pollution Bulletin 50: 787–789.

Boström, C., E. Bonsdorff, P. Kangas & A. Norkko, 2002. Long-term changes of a brackish-water eelgrass (*Zostera marina* L.) community indicate effects of coastal eutrophication. Estuarine, Coastal and Shelf Science 55: 795–804.

Cardoso, P. G., A. Brandão, M. A. Pardal, D. Raffaelli & J. C. Marques, 2005. Resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances. Marine Ecology Progress Series 289: 191–199.

Cardoso, P. G., M. A. Pardal, A. I. Lillebø, S. M. Ferreira, D. Raffaelli & J. C. Marques, 2004. Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. Journal of Experimental Marine Biology and Ecology 302: 233–248.

Clarke, K. R., 1990. Comparison of dominance curves. Journal of Experimental Marine Biology and Ecology 138: 130–143.

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

Dauer, D. M., M. W. Luckenbach & A. J. Rodi Jr., 1993. Abundance biomass comparison (ABC method): effects of an estuarine gradient, anoxic/hypoxic events and contaminated sediments. Marine Biology 116: 507–518.

Diaz, R. J., M. Solan & R. M. Valente, 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. Journal of Environmental Management 73: 165-181.

Dolbeth, M., M. A. Pardal, A. I. Lillebø, U. Azeiteiro & J. C. Marques, 2003. Short- and long-term effects of eutrophication on the secondary production of an intertidal macrobenthic community. Marine Biology 143: 1229–1238.

Margalef, R., 1968. Perspectives in Ecological Theory. University of Chicago Press, Illinois.

Marques, J. C., P. Maranhão & M. A. Pardal, 1993. Human impact on the subtidal macrobenthic community structure in the Mondego estuary (Western Portugal). Estuarine, Coastal and Shelf Science 37: 403–419.

Marques, J. C., S. N. Nielsen, M. A. Pardal & S. E. Jørgensen, 2003. Impact of eutrophication and river management within a framework of ecosystem theories. Ecological Modelling 166: 147–168.

McLusky, D. S., 1990. The Estuarine Ecosystem. 2nd edn. Chapman & Hall, New York.

Molvær, J., J. Knutzen, J. Magnusson, B. Rygg, J. Skei & J. Sørensen, 1997. Classification of environmental quality in fjords and coastal waters. SFT Guidelines 97:03. Norwegian Pollution Control Authority, Oslo.

Muxika, I., A. Borja & W. Bonne, 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological Indicators 5: 19–31.

NEAGIG Invertebrate Group, 2004. Minutes of Northeast Atlantic Geographical Intercalibration Group. Benthic Expert Meeting. Kristineberg Marine Station, Sweden.

Pardal, M. A., P. G Cardoso, J. P. Sousa, J. C. Marques & D. Raffaelli, 2004. Assessing environmental quality: a novel approach. Marine Ecology Progress Series 267: 1–8.

Prior, A., A. C. Miles, A. J. Sparrow & N. Price, 2004. Development of a classification scheme for the marine benthic invertebrate component, water framework directive. Phase I & II. Transitional and coastal waters. R & D Interim Technical Report.

Rodrigues, E., 2004. Variação das comunidades macrobentónicas subtidais do estuário do Mondego ao longo de mais de uma década. (Master Thesis). Universidade de Coimbra, Coimbra.

Ros, J. D. & M. J. Cardell, 1991. La diversidad específica y otros descriptores de contaminación orgánica en comunidades bentónicas marinas. Actas del symposium sobre diversidad biológica. Centro de Estudios Ramón Areces, Madrid, 219–223.

Schlacher, T. A. & T. H. Wooldridge, 1996. How sieve mesh size affects sample estimates of estuarine benthic macrofauna. Journal of Experimental Marine Biology and Ecology 201: 159–171.

Shannon, C. E. & W. Weaver, 1963. The Mathematical Theory of Communication. The University of Illinois Press, Illinois.

Strasser, M., R. Dekker, K. Essink, C.-P. Günther, S. Jaklin, I. Krönke, P. B. Madsen, H. Michaelis & G. Vedel, 2003. How predictable is excellent bivalve recruitment in the Wadden Sea after a severe winter? Journal of Sea Research 49: 47–57.

Thompson, B. W., M. J. Riddle & J. S. Stark, 2003. Cost-efficient methods for marine pollution monitoring at Casey Station, East Antarctica: the choice of sieve mesh-size and taxonomic resolution. Marine Pollution Bulletin 46: 232–243.

Verdelhos, T., J. M. Neto, J. C. Marques & M. A. Pardal, 2005. The effect of eutrophication abatement on the bivalve *Scrobicularia plana*. Estuarine, Coastal and Shelf Science 63: 261–268.

Warwick, R. M., 1986. A new method for detecting pollution effects on marine macrobenthic communities. Marine Biology 92: 557–562.

Warwick, R. M., 1993. Environmental impact studies on marine communities: pragmatical considerations. Australian Journal of Ecology 18: 63–80.

Warwick, R. M. & K. R. Clarke, 1994. Relearning the ABC: taxonomic changes and abundance/biomass relationships in disturbed benthic communities. Marine Biology 118: 739–744.

Water Framework Directive, 2000. European Communities Official Journal L327, 2000/60/EC: 1-72.

Wilson, J. G. & B. Elkaïm, 1992. Estuarine bioindicators: a case for caution. Acta Oecologica 13: 345–358.

Ysebaert, T., P. M. J. Herman, P. Meire, J. Craeymeersch, H. Verbeek & C. H. R. Heip, 2003. Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde estuary, NW Europe. Estuarine, Coastal and Shelf Science 57: 335–355.

Zacharias, M. A., M. C. Morris & D. E. Howes, 1999. Large scale characterization of intertidal communities using a predictive model. Journal of Experimental Marine Biology and Ecology 239: 223–242.