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**The robustness of ecological indicators to detect long-term changes in
the macrobenthos of estuarine systems.**

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Abstract

Accurate and reliable benthic quality indicators are in great demand following the
recent developments and the strict time schedule for implementing the European Water
Framework Directive. The Mondego estuary has experienced a progressive deterioration
during the 1990's, followed by a partial ecological recovery due to restoration measures in
1997/1998. We have used the estuary as a model system to test the performance and
robustness of a set of ecological indicators in highlighting the changes in the ecological
state of intertidal areas. Over a period of 17 years (1985-2002), we calculated Margalef,
Shannon-Wiener, Berger-Parker, Taxonomic Distinctness measures, AZTI's Marine Biotic
Index, Infaunal Trophic Index, and Eco-Exergy based indices and tested differences
across periods characterised by different anthropogenic disturbance. We combined
temporal data within three periods: before, during and after disturbance, based on

progressive information on the changes in the extended type of anthropogenic disturbance. Indices were then compared with biological and abiotic descriptors (macroalgae, macrophytes, benthic macrofauna, nutrients concentration, sediment grain size and total organic carbon). We found great disparity in the indicators ability to capture temporal changes, showing distinct performances at each site. At the *Z. noltii* site, only Margalef, Total Taxonomic Distinctness and the thermodynamically based indices captured temporal changes, despite giving higher values during the disturbance period. At the bare sediment site, Taxonomic Distinctness, ITI, Shannon-Wiener, Berger-Parker, AMBI and the TBI were able to distinguish between periods, in agreement with the differences observed analysing the macrobenthic assemblages. Furthermore, Taxonomic Distinctness was not robust enough to detect any temporal or spatial change. We thus suggest further research to understand the behaviour of ecological indicators, in view of their crucial importance for the management and protection of marine coastal areas.

Keywords: ecological indicators, benthic macrofauna, intertidal, ecological status, estuaries, Portugal

1. Introduction

1.1. Background

Ecological indicators are commonly used to provide synoptic information about the state of ecosystems. Most often they summarise information on the structure and/or functioning of ecosystems using a particular biological aspect or component of these ecosystems, such as diversity, productivity or environmental conditions (Salas et al.,

2006). In general, the application of ecological indicators is not exempt from criticism. Aggregation of different metrics results in oversimplification of the ecosystem under observation. Moreover, the choice of which ecological indicators to use should follow criteria based on the question we want to address and the theoretical background on which the indicator was created. Before being able to use an indicator it is thus necessary to establish its applicability and robustness for a set of conditions, by testing its consistency with the ecological expectation.

The implementation of the European Water Framework Directive (WFD) (EC, 2000), as well as the recent European Marine Strategy Directive 2008/56/EC (EC, 2008) require the use of well-founded ecological indicators, which has contributed greatly to develop this research field and stimulate analyses to establish indicators' applicability to a variety of ecosystems. Indeed, since 2000, numerous ecological quality assessment tools have been proposed by the European scientific community (e.g. Borja et al., 2004; Rosenberg et al., 2004; Bald et al., 2005; Simboura et al., 2005; Devlin et al., 2007; Muxica et al., 2007; Pinto et al., 2009). The WFD suggests that the water body ecological quality should be primarily determined by biological quality elements and then by hydromorphological and physicochemical quality elements.

The composition and structure of benthic macrofauna is one of the indicated biological quality elements to be used in transitional and coastal waters for quality status assessment. Benthic communities may detect the level of stress locally and integrate the recent history of stress, constituting a sort of memory for the system. Macrofauna are dominated by species with different mobility, life-cycle and tolerance to stress (Dauer, 1993), which covers the WFD demand of integrating differently sensitive species. The response of macrobenthic communities to several types of stress is well studied, based on multivariate analyses that take into account variations in species diversity and their relative

abundance between perturbed and control sites (e.g. Pearson and Rosenberg, 1978, Warwick and Clarke 1993, Gray 2002). Based on this knowledge it is possible to determine *a priori* the ecological indicator behaviour and thus its appropriateness to detect changes in the variable of interest. The aim of our study was to test the performance and robustness of a large set of ecological indicators based on the composition and structure of macrobenthic communities.

A plethora of methodologies with hundreds of indices, metrics and evaluation tools are presently available (Borja & Dauer, 2008; e.g. see summary in Diaz et al., 2004). An ecologically parsimonious approach dictates that investigation should place greater emphasis on evaluating the suitability of indices that already exist prior to developing new ones (Díaz et al., 2004). We thus selected a large set of ecological indicators of different types (Margalef, Shannon-Wiener, Berger-Parker, Taxonomic Distinctness measures, AZTI's Marine Biotic Index (AMBI), Word Infaunal Trophic Index (ITI), and Eco-Exergy based indices) and compared the status of two sites "Z. *noltii* site" and a "bare sediment" in the Mondego estuary (western coast of Portugal; 40°08'N, 8°50'W) over a period of seventeen years. During this period, the estuary experienced changes in the type and severity of anthropogenic stress, which changed macrophyte distribution and macrofauna communities. We asked whether different ecological indicators were able to detect changes and whether these changes were in agreement with the differences observed analysing species composition of macrobenthic assemblages. Specifically, we tested for differences in the macrofauna composition and biological indicators between the periods during and after the major anthropogenic changes. We also assessed which ecological indicators were more robust in detecting these changes.

1.2. Synopsis of human induced changes in the Mondego estuary ecosystem

The Mondego estuary is a relatively small (860 ha) warm-temperate intertidal system. At present, the entire catchment area contributes a large loading of nutrients and several chemicals into the system. Moreover, the estuary supports industrial activities, salt-extraction, and aquaculture farms, and is located close to a tourism centre (Figueira da Foz). The estuary's terminal part is 7 km long and is 2-3 km across at its widest part, consisting of two arms, separated by the Murraceira Island (Fig. 1). The northern arm is deeper (5-10 m during high tide), constituting the main navigation channel and the harbour location. The southern arm is shallower (2-4 m during high tide), and is characterised by large areas of intertidal flats exposed during low tide (almost 75% of the total area of the southern arm). A detailed description of the system can be found in Cardoso et al. (2004) and Marques et al. (2007). A comprehensive study on the environmental quality of the Mondego estuary was carried out during the last two decades (Marques et al., 2007) and covered several quality elements (water quality, hydraulics and sediments dynamics, benthic intertidal and subtidal communities, *Z. noltii* beds and green macroalgal cover). Such analyses allowed detection increased eutrophication symptoms and ecological deterioration in the South arm since early 1990s, when the communication between the two arms was interrupted, increasing water residence time and nutrient concentration (Lillebø et al., 2005).

Since then, macroalgal blooms became more frequent and intense (mostly *Ulva* spp.) (e.g. Martins et al., 2001; Cardoso et al., 2004; Pardal et al., 2004; Leston et al., 2008) and have affected *Z. noltii* beds, reducing the area covered by the seagrass and the biodiversity and community biomass in the remaining meadow (Cardoso et al., 2008; Lillebø et al., 2005). Before the interruption, the *Z. noltii* covered 15 ha (in 1986), which decreased to 1.6 ha in 1993, when macroalgal blooms increased up to 415 g m² (Fig. 2). In 1997 *Z. noltii* bed reached its lowest value (0.02 ha). Through time, such modifications promoted the emergence of a simplified trophic structure, which has been analysed in the existing

literature (e.g. Dolbeth et al., 2007; Martins et al., 2005; Lopes et al., 2006; Patrício and Marques, 2006). Since 1998, the system has started recovering, after the decrease in freshwater discharge from the Pranto River and the limited re-establishment of communication between the two arms (Marques et al., 2007; Leston et al., 2008; Cardoso et al., 2008). *Z. noltii* meadow has begun to recover and in 2005, it occupied an area of about 4.2 ha.

2. Material and Methods

2.1. Description of the database used

Intertidal benthic communities were first sampled in 1985 (during winter) and 1986 (during summer). Subsequently, the community was regularly monitored from January 1993 to January 1996, and again from January 1999 to December 2002 once every two weeks from 1993 to 1996, and monthly from 1999 to 2002.

In all cases, sampling was conducted at two sites in the South arm (Fig. 1), within *Z. noltii* meadow, which persisted during early 1990s, and on an area where the *Z. noltii* has disappeared, now characterised by bare sediment. Each time and at each site, six replicate cores were taken to a depth of 20 cm (13 cm inner diameter and then pooled together for macrofauna analyses. Sediment was washed through a 500 µm mesh sieve and animals preserved in 4% buffered formalin. Animals were identified to species level and subsequently dried at 70°C for 72h to estimate biomass as dry weight (DW) and ash free dry weight (AFDW), after combusting samples for 8h at 450°C.

2.2. Hydrological changes and variation in macroalgal and macrophyte coverage and biomass

Hydrologic changes in the Mondego estuary, following human interventions, have been assessed using technical reports, maps and aerial photographs. Samples of macrophytes and macroalgae were taken once every two weeks from January 1993 to January 1996, and subsequently, from January 1999 to December 2002, with a monthly periodicity. Six cores (13 cm diameter to a depth of 20 cm) were randomly taken from each site (*Z. noltii* site and bare sediment site), during low tide. Macroalgal and macrophyte species were identified to the species' level, dried at 70°C for 48 hours and then combusted at 450°C for 8 hours to estimate biomass as AFDW. Additionally, the area of *Z. noltii* meadows was measured through aerial photographs and field campaigns (Marques et al., 2007).

2.3. Ecological indicators

2.3.1. Description and calculation

The following ecological indicators were calculated using the macrofauna database, for each site and time. Replicates within site and time were pooled.

1. The **AZTI's Marine Biotic Index, AMBI**, (Borja et al., 2000) is based on the proportion of five ecological groups to which the benthic species are allocated. The soft bottom macrofauna was divided into five groups according to their sensitivity as a function of an increasing stress (see Table 1 for details). The AMBI 4.0 software, freely available at <http://www.azti.es>, was used to calculate this index, using a species list of July 2006 and following Borja and Muxika (2005) guidelines. AMBI ranges from 0 to 7, and a better quality trend would be indicated by a decrease on the index value (see Table 1).

2. To estimate Taxonomic Diversity indices – **Taxonomic Diversity**, Δ ; **Taxonomic Distinctness**, Δ^* and **Total Taxonomic Distinctness**, $s\Delta^+$ – a hierarchical Linnean classification was used as a proxy for cladograms representing the relatedness of individual species. For each location, a composite taxonomy was compiled, five taxonomic levels were considered (species, genus, family, order, class and phylum) (Clarke and Warwick, 1998, 2001a) and the indices were calculated using PRIMER 5 (Software package from Plymouth Marine Laboratory, UK) (Table 1). Higher values of these indices suggest that, on average, the species in the assemblage are not closely related, being higher biodiversity.
3. The **Berger-Parker**, **Margalef** and **Shannon-Wiener** indices were also calculated (see Table 1 for details). The Berger-Parker takes values from 0 to 1, with higher values corresponding to lower diversity. An increase on Margalef index is generally accepted as an indication of quality improvement. The $\log_2$ was used to compute the Shannon-Wiener; therefore the results were expressed in bits/individual and higher values correspond to higher diversity.
4. The **Infaunal Trophic Index**, **ITI**, was calculated following Codling and Ashley (1992). For each sample, the taxa contributing to the top 80% of the abundance have been allocated to trophic groups. In addition, in samples with ten or less identified taxa, all were taken into consideration for calculation purposes. Once generated, the list of the unclassified taxa was compared with the lists provided by Word (1979, 1990). Table 2 shows the Mondego estuary species' trophic classification. Values of the index vary from 0 to 100 with low values indicating degraded conditions. ITI values were used to classify areas of seabed into either "normal" (ITI values 100-60), "modified" (60-30) or "degraded" (30-0) (Bascom et al., 1978).

5. **Exergy** expresses the work capacity relative to the same system but at thermodynamic equilibrium with its environment, which means that all the components would be inorganic at the highest possible oxidation state and homogeneously distributed at random in the system (no gradients). Of course exergy cannot be measured precisely, as the total internal energy content of a body cannot be measured exactly. Nevertheless, with regard to far from equilibrium systems (e.g. ecosystems), a reasonable approximation can be obtained if exergy is calculated only from the chemical potentials (which are in fact extremely dominant in ecosystems) (Jørgensen, 2000). This approximate measure can be called Eco-Exergy index (Marques et al., 1997). If the total biomass in the system remains constant through time, then the variation of Eco-Exergy will be a function of only the quality of the biomass (structural complexity) or, in other words, of the information embedded in the biomass, which may be called **Specific Eco-Exergy**. Both indices were calculated using macrofauna biomass data and β -values given by Jørgensen et al. (2005) according to the algorithm shown in Table 1.

2.3.2. Criteria used to assess the ecological indicators' success/failure

According to Borja et al. (2008), index validation should ideally include (1) testing of the index using an independent data set, different from the index development data set; (2) setting *a priori* correct classification criteria and/or (3) presentation of a strong *a posteriori* justification for use based upon best professional judgment. The first requirement was fulfilled using our own data set from two intertidal sites in the Mondego estuary. In order to assess the indices success or failure to discriminate the ecological quality status, two main criteria were used:

- 1) The classification proposed by the index authors. High values of Margalef, Shannon-Wiener, Taxonomic distinctness indices, ITI and the thermodynamically based indices (TBI) indicate high ecological quality status. High values of Berger-Parker and AMBI indicate low ecological quality status (Table 1).
- 2) Determining the level of agreement provided by an index with best professional judgment. In our case study, the indices scores were compare with the existing knowledge about the ecological changes of the system (e.g. Marques et al., 1993, 2007; Pardal et al., 2000, 2004; Dolbeth et al., 2007; Cardoso et al., 2004, 2008; Lillebø et al., 2005, 2007; and the present study) according to ultimate causal stressors that had induced such changes (e.g. hydrology and eutrophication) and to other potential stressors (proximate causes) generated by changes in hydrology and eutrophication (e.g. changes in seagrass cover and biomass (*Z. noltii* site); changes in the algal mats biomass (bare sediment site); nutrients levels; sediment grain size and total organic carbon (TOC).

2.4. Statistical analysis

2.4.1. Univariate analysis

One-way ANOVA, with time as fixed factor (2 levels: “during the interruption of flow between the North and South arms – 1993 to 1995” and “after the experimental opening of the connection between the two arms of the estuary- 2000 to 2002”) and 30 and 29 replicates for *Z. noltii* site and bare sediment site, respectively, was used to test for temporal differences in each of the two sites (with respect to total abundance, total biomass, number of species, Margalef, Shannon-Wiener, Berger–Parker, Biotic Coefficient (AMBI), Taxonomic Distinctness indices, ITI, Eco-Exergy and Specific Eco-Exergy). The

“before the interruption- 1985-1986” period was left out from this analysis due to its small number of replicates. Data were checked for normality using the Kolmogorov-Smirnov test and homogeneity of variances was assessed by the Levene’s test. Data were transformed when homoscedasticity assumption was not met. The analyses were performed using the software package Minitab version 12.2. In each site, we expected no differences during the years of interruption, which strongly increased eutrophication effects and sedimentation, thus affecting *Z. noltii* and the post-management years.

2.4.2. Multivariate analysis

In order to examine the benthic macroinvertebrate communities’ temporal dynamics in Mondego estuary South arm, a multivariate analysis was performed on biological data from the *Z. noltii* site and bare sediment site using the PRIMER 5.2.6 © software (Software package from Plymouth Marine Laboratory, UK). Data of abundance were square root transformed prior to analysis in order to scale down the effects on the ordination of highly abundant species (Clarke and Warwick, 2001b). The Bray Curtis similarity matrix was calculated and used to generate a 2-dimensional plot with the non-metric multidimensional scaling (nMDS) technique (Clarke, 1993). The one-way ANOSIM test was performed to test for differences among time periods. Species contributing 70% to differences between periods were identified using similarity percentages (SIMPER; Clarke, 1993).

3. Results

3.1. Changes in macrofauna assemblages

In both sites, there was a significant decline in total macrofauna abundance in the “after the experimental opening” period (1-way ANOVA, *Z. noltii* site: $F_{1,58} = 12.59$, $p < 0.01$; bare sediment site: $F_{1,56} = 24.06$, $p < 0.01$) (in seagrass bed, from 1993 to 1995: 35181 - 249966 ind m^{-2} ; from 2000 to 2002: 5785 - 120375 ind m^{-2} ; in the bare sediment site, from 1993 to 1995: 6515 - 167554 ind m^{-2} ; from 2000 to 2002: 3430 - 28324 ind m^{-2}) (Fig. 3A). Whereas the total biomass, in the *Z. noltii* site, was significantly higher “during the interruption” period (1-way ANOVA, $F_{1,58} = 7.63$, $p < 0.01$), ranging from 58 to 158 g m^{-2} . In the bare sediment site, the total biomass was significantly higher after the implementation of preliminary management measures (range 20-75 g m^{-2}) (1-way ANOVA, $F_{1,56} = 20.59$, $p < 0.01$) (Fig. 3B). In both sites, more species were present “during the interruption” period compared to “after the experimental opening” period. (Fig. 3C) (1-way ANOVA, *Z. noltii* site: $F_{1,58} = 77.23$, $p < 0.01$; bare sediment site: $F_{1,56} = 15.65$, $p < 0.01$). Generally, the *Z. noltii* site supported more species than the bare sediment site (in the *Z. noltii* site, from 1993 to 1995: 14-40 species; from 2000 to 2002: 9-25 species; in the bare sediment site, from 1993 to 1995: 7-28 species; from 2000 to 2002: 7-20 species).

In both sampling sites, the results of the one-way ANOSIM test showed significant differences ($p < 0.001$) between the two periods. Nonetheless, the *Z. noltii* site had higher Global R value than the bare sediment site (Global $R = 0.42$ and 0.32, respectively). The nMDS showed that the “after the experimental opening” period is closer to the reference points, particularly in the *Z. noltii* site (Fig. 4).

The SIMPER analysis showed that in the *Z. noltii* site, *Hediste diversicolor*, *Hydrobia ulvae*, *Scrobicularia plana*, *Cyathura carinata*, Tubificidae, *Alkmaria romijni*, *Heterosmastus filiformis*, *Capitela capitata*, *Cerastoderma edule* and *Chaetozone setosa* were the species responsible for the separation between periods (Table 3). During the period of works and closure of the channel, the abundance of some polychaete species

(*A. romijni*, *H. filiformis*, *C. capitata* and *C. setose*), of Tubificidae and *H. ulvae* dramatically increased as compared to the period before disturbance (1986) and also to the period following mitigation measures (1998-2002) (Table 3).

3.2. Ecological indicators

In both sites, Margalef (Fig. 5A) exhibited significant differences between the two time periods considered (1-way ANOVA, *Z. noltii* site: $F_{1,58} = 58.70$, $p < 0.01$; bare sediment site: $F_{1,56} = 12.29$, $p < 0.01$), presenting unexpected higher values “during the interruption” period. Overall, Margalef was always higher in the *Z. noltii* site than the bare sediment site. The qualitative measure based on the presence/absence of species, TTD (Fig. 5B), showed similar behaviour (1-way ANOVA, *Z. noltii* site: $F_{1,58} = 76.82$, $p < 0.01$; bare sediment site: $F_{1,56} = 15.12$, $p < 0.01$).

In contrast to the diversity indices of Margalef and TTD, there were no difference in the Taxonomic Distinctness between periods (Fig. 5C), ($p > 0.05$), and the index was not larger in the *Z. noltii* compared to the bare sediment site.

The Taxonomic Diversity (Fig. 5D) and ITI (Fig. 5E) were unable to detect temporal changes in the *Z. noltii* site, but, as theoretically expected, exhibited lower values “during the interruption” period at the bare sediment site (1-way ANOVA, $F_{1,56} = 10.67$, $p < 0.01$ and $F_{1,56} = 6.49$, $p < 0.01$, respectively). Both indices were lower at *Z. noltii* site, differing from the behaviour that we could predict based on the authors classification and our own knowledge regarding the system. According to Bascom et al. (1978), ITI classified the benthic community in the bare sediment site community as a “slightly modified” to “normal”.

The Shannon-Wiener (Fig. 5F) values were higher in the bare sediment site, because of the dominance of *H. ulvae* in the *Z. noltii* site (almost 90% of the total abundance).

While no significant differences were detected between the pre- and post-management periods in the reference site (1-way ANOVA, $F_{1,58} = 0.14$, $p = 0.71$), in the bare sediment site, the values were significantly higher “after the experimental opening” period (1-way ANOVA, $F_{1,56} = 9.68$, $p < 0.01$). As expected, the Berger-Parker dominance (Fig. 5G) showed the opposite behaviour, with higher values in the *Z. noltii* site, again due to *H. ulvae*’s dominance. In the bare sediment site, the dominance significantly decreased after the implementation of the management plan (1-way ANOVA, $F_{1,56} = 12.82$, $p < 0.01$).

A significantly higher AMBI value (Fig. 5H) was found “during the interruption” period at the reference site (1-way ANOVA, $F_{1,58} = 5.91$, $p < 0.02$), indicating worse ecological conditions during this time period. In the bare sediment site, no differences regarding the pre- or post restoration periods were detected (1-way ANOVA, $F_{1,56} = 2.53$, $p > 0.05$). This index showed similar values in both sites.

In the *Z. noltii* site, Eco-Exergy and Specific Eco-Exergy (Fig. 5I and J) were significantly higher “during the interruption” period (1-way ANOVA, Eco-Exergy: $F_{1,58} = 8.66$, $p < 0.01$; Specific Eco-Exergy: $F_{1,58} = 56.85$, $p < 0.01$). In the bare sediment site, these indices showed the opposite behaviour, with significantly higher values “after the experimental opening” period (1-way ANOVA, Eco-Exergy: $F_{1,56} = 25.24$, $p < 0.01$; Specific Eco-Exergy: $F_{1,56} = 4.8$, $p < 0.03$), indicating higher biomass structure and information embedded in it. The indices had higher values in the *Z. noltii* site than in the bare sediment site, following the behaviour that we would predict based on *a priori* established criteria.

4. Discussion

4.1. Ecological indicators performance

Our analyses on the long-term changes in the composition of macrofauna assemblages confirmed the previous studies (e.g. Dolbeth et al., 2007; Marques et al., 2007; Cardoso et al., 2008) and highlighted that the system seems to be recovering from the environmental changes occurred during the 1990s. At both sites, during the 1993-1995, the total dissolved inorganic nitrogen in the water column was higher than “after the experimental opening period” (Leston et al., 2008), the macroalgae assemblages were dominated by Ulvaceans, the *Z. noltii* cover and biomass reached their lowest values (Marques et al., 2007) and the abundance of the opportunistic species increased. After 1997/1998, the macrofauna and macrophyte assemblages showed a partial recovery towards the reference situation.

The selected ecological indicators are based upon community characteristics that are typically part of, or inherent to, the diversity of definitions of biotic integrity that include elements of species diversity, abundance, energy-flow-food web structure, maintenance of complexity and self-organization (Borja and Dauer, 2008). Therefore, according to the classification proposed, we would expect significantly higher values for Margalef, Shannon-Wiener, Taxonomic Distinctness measures, ITI, Eco-Exergy and Specific Eco-Exergy; AMBI and Berger-Parker should present significant lower values during the post-management period, at both sites. In the *Z. noltii* site, only AMBI reflected the ecological expectation, of a community with higher abundance of opportunist species “during the interruption” period. Margalef, TTD and the TBI indicated higher diversity and biomass structure/information “during the interruption” period, indicating that this community was apparently able to obtain some benefit from the existing conditions (higher nutrient input, lower water residence time). In the bare sediment site, with the exception of Margalef and TTD, all ecological indicators able to detect significant differences between time periods,

performed as initially projected, indicating a better ecological status “after the experimental opening” period.

Furthermore, even if hydrology and eutrophication (both water column related), were probably the major drivers “shaping” South arm environmental conditions, the two study sites were differently affected by distinct stressors resulting from these drivers. The bare sediment site, characterised by sandy sediments, which have not supported rooted macrophytes for more than 15 years, lower organic matter content (mean $3.0\% \pm 1.14$) and lower water-flows ($0.8\text{--}1.2 \text{ m s}^{-1}$) has been regularly covered by extensive algal mats (e.g. Lillebø et al., 2005; Dolbeth et al., 2007; Marques et al., 1997, 2003, 2007; Cardoso et al., 2008). These macroalgal blooms have well-documented effects, causing dramatic changes in sediment chemistry, nutrient release, rates of oxygen consumption and accumulation of metal sulfides and H_2S in sediment pore waters. These shifts are profound changes in habitat quality for invertebrate assemblages (e.g. Pearson & Rosenberg, 1978; Raffaelli et al 1998; Cloern, 2001; Patrício & Marques, 2006). On the other hand, the *Z. noltii* site, represents a distinct complex and heterogeneous habitat with particular physical and chemical conditions, characterised by muddy sediments covered with rooted macrophytes, higher organic matter content (mean $6.2\% \pm 1.76$), higher water-flow velocity ($1.2\text{--}1.4 \text{ m s}^{-1}$) (e.g. Dolbeth et al., 2007) and supporting a higher diversity of species (Cardoso, 2004). Knowing that the direct and indirect responses to changes in hydrology and nutrient enrichment are expressed in varying degrees in different habitats (Cloern, 2001), and taking into consideration the effect of site-specific operative stressors, we have considered *Z. noltii* site as representing a better ecological condition. Therefore, we would expect higher values for Margalef, Shannon-Wiener, Taxonomic Distinctness measures, ITI, Eco-Exergy and Specific Eco-Exergy in the reference site; AMBI and Berger-Parker would present the opposite behaviour. In fact, only Margalef, TTD and TBI

followed the behaviour that we could predict based on our knowledge of the system (Fig. 4). Taxonomic Distinctness and AMBI presented similar values at both sites, indicating that these two metrics may be of limited efficacy in understanding the environmental conditions being investigated. AMBI values were dominated by *H. ulvae* abundance. As numerous estuarine species, this gastropod belongs to ecological group III, which comprises species tolerant to an excess of organic matter enrichment, a widespread stressor in transitional systems. Such explanation is supported by considering that Shannon-Wiener and Berger-Parker indices indicated that the *Z. noltii* site was of lower quality than the bare sediment. These indices are based on measures of equitability and dominance, and can vary unexpectedly if one species becomes dominant, as is the case of *H. ulvae*. Indeed, excluding *H. ulvae* from the dataset resulted in opposite results. *H. ulvae* is a very mobile species that may migrate during low tide towards areas more protected and with high food availability, as is the case of *Z. noltii* site. Therefore, more attention should be paid to the ecology and distribution of species to decrease the probability of giving an erroneous classification based on a species with high mobility. Furthermore, it should be noticed that in estuaries species dominance occurs more often than on coastal systems, even in relatively pristine conditions, due the large natural variability of environmental parameters. According to Constanza et al. (1992) and Elliot and Quintino (2007), in these ecosystems, the “positive effect” of natural environmental variability is shown as the ability of some organisms, which can tolerate the adverse and variable environmental conditions, to capitalise on the lack of inter-specific competition and thus achieve high population densities. Therefore, caution should be taken when interpreting equitability and dominance results based in estuarine macrobenthic assemblages.

Following Word (1990), ITI showed a higher value in the bare sediment site, where the sediment organic matter content was lower. However, in the *Z. noltii* site the sediment

organic matter has a natural source (macrophyte primary production). For that reason, the metric is not correctly capturing the environmental conditions being investigated. Moreover, ITI is difficult to apply in environmental studies once determining the organisms' diet may become reasonably problematic. Generally, the real diet (studied observing stomach content) is difficult to establish, and can vary from one population to another among the same taxonomic entity (e.g. *H. filiformis* was classified by Brown (1985) as subsurface deposit feeder and as surface detritus feeder by Word (1990)).

According to our study, it seems that the ecological indicators response was not always temporally and spatially consistent (Table 4). These results are quite interesting, raising further questions.

It is well known, that estuaries are particularly challenging due to strong spatial, seasonal and inter-annual variations of environmental characteristics that influence benthic communities (Chainho et al., 2007). Apart from salinity regime, plant presence/absence, sediment grain size and organic matter content, the hydrological conditions, such as flood and drought periods, may have great impact in communities and thus on diversity measures (Pearson and Rosenberg, 1978; Gray, 1997; Raffaelli et al., 1998; Cloern, 2001; Salas et al., 2006; Chainho et al., 2007; Dolbeth et al., 2007; Cardoso et al., 2008; Teixeira et al., 2008).

In summary, we may say that although hydrology and eutrophication were the major drivers, other ultimate causal factors, probably also played a role during the study period, making it difficult to unambiguously distinguish the anthropogenic disturbance effects from the natural temporal wide-scale variability. In any case, the ecological indicators' results were constrained to the type and magnitude of the drivers' combined effect that occurred at either or both sites during the study period. Therefore, caution must be taken interpreting values isolated from the ecological community context.

4.2. *Scientific implications and limitations of the study*

This paper laid out some of the history of anthropogenic modifications and sketched a brief image of the ecological changes over the considered timeframe (derived partially from previously reported literature). In addition, many readers have pondered comparisons among all of the new indices that seem to continuously emerge (e.g. Díaz et al., 2004; Labruno et al., 2005; Aubry & Elliot, 2006; Quintino et al., 2006; Dauvin et al., 2007; Blanchet et al., 2008). The present study compared several ecological indicators (all using benthic macrofauna) to the image of change that can be cast through a set of measurements on alterations in water quality, hydraulic/sediments, benthic invertebrate and plant/algal communities, and spatial distribution. Even if this broad comparison has been made in the past, given the European initiatives to use indices to monitor estuarine system health, we hope this study could be a useful and directly applicable demonstration of the care which must be taken in selecting appropriate indices. Indeed, it is very challenging to see such different predictions from the diverse indices, given that the same data were used (albeit in different ways, but that was the aim). Blanchet et al. (2008), testing five biotic indices (AMBI, BENTIX, Shannon-Wiener diversity, BQI and BOPA) in two semi-enclosed, sheltered coastal ecosystems and in one transitional water body situated along the Western French coast, also found that these five indices rarely agreed with each other, describing very different pictures of the overall ecological quality status of the three study areas. A similar conclusion was made by Chainho et al. (2007) studying the Mondego estuary subtidal assemblages, using four indices (Margalef, Shannon-Wiener, AMBI and W-Statistic).

Notwithstanding, it is obvious for us, that the perfect way to compare indices would be to (ideally) set up an ecological situation, in as controlled a manner as possible, in which one knew the “precise, accurate and totally clear answer” already and where strong putative gradients were selected for validation testing (e.g. Borja et al., 2000; Quintino et al., 2006). Nevertheless, the available ecological tools will have to work in “existent” ecosystems and available datasets. In our case study the ecological indicators diagnostic ability was only tested in two sites: a “*Z. noltii* site” and a “bare sediment site” during two different time periods, characterised by different levels of impact. We did not cover the five categories featured within the WFD (bad, poor, moderate, good and high). Therefore, this study only addresses two conditions and for that reason provides a limited assessment. Clearly, with other case-studies, further steps will have to be done to fulfil the WFD requirements. The second phase of the European WFD Intercalibration exercise will certainly be critical, particularly for transitional systems. Even so, it is our conviction that initially the proposed ecological indicators should be able to give the correct assessment in this case, particularly, when some of these metrics are being selected and combined into multimetric indices used to assess the ecological quality status all over Europe with obvious legal implications. We truly agree that without a sound and obvious ecological foundation, an index will not be policy-relevant and therefore difficult to use to make policy choices (Borja and Dauer, 2008), thus the results must be justifiable and scientifically defensible. Experiences gather from different ecosystems, distinct geographical ranges, various hydrological regimes, and diverse pressure types are vital and urgent.

5. Conclusions

Relevant conclusions can be taken from our study: (1) Taxonomic Distinctness did not detect temporal and spatial changes; (2) ITI did not detect spatial changes largely because estuarine macrofauna shows diet plasticity and is able to adapt its trophic role; (3) The equitability and dominance measures were totally influenced by the presence of a common species with high mobility, (4) The best distinction between sites was given by Margalef, TTD and TBI, (5) The indicators ability to capture temporal changes was different in each site, revealing habitat-dependency and (6) although some ecological indicators have been effective in differentiating the two time periods, not all have behaved consistently with ecological expectation. These considerations are crucial for management, once an indicator is expected to respond to a particular pressure and lead to a human response to reduce environmental impacts through the systems' driving forces, with all the social and economic repercussions of that action.

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717 Table 1. Ecological indicators: algorithms and ecological classification.

Indicator	Algorithm/ Explanation	Classification
1. Species as indicators		
AZTI's Marine Biotic Index (Borja et al. 2000)	$AMBI = \frac{\{(0 \times \%GI) + (1.5 \times \%GII) + (3 \times \%GIII) + (4.5 \times \%GIV) + (6 \times \%GV)\}}{100}$ <p>I. Species very sensitive to organic enrichment and present under unpolluted conditions; II. Species indifferent to enrichment, always in low densities with non-significant variations with time; III. Species tolerant to excess of organic matter enrichment; IV. Second-order opportunist species, mainly small sized polychaete; V. First-order opportunist species, essentially deposit-feeders</p>	<p>Normal 0-1.2 Slightly polluted 1.2-3.2 Moderately polluted 3.2-5.0 Highly polluted 5.0-6.0 Azotic 6.0-7.0</p>
2. Diversity		
Average Taxonomic Diversity (Warwick and Clarke 1995)	$\Delta = \left[\sum_{i < j} \omega_{ij} x_i x_j \right] / [n(n-1)/2]$	
Average Taxonomic Distinctness (Warwick and Clarke 1995)	$\Delta^* = \left[\sum_{i < j} \omega_{ij} x_i x_j \right] / \left[\sum_{i < j} \delta_{ij} x_i x_j \right]$	Higher value, higher biodiversity – on average species in the assemblage are not closely (phylogenetic) related
Total Taxonomic Distinctness (Warwick and Clarke 1995)	$s\Delta^+ = \left[\sum_i \left(\left(\sum_{j \neq i} \omega_{ij} \right) / (s-1) \right) \right]$ <p>x_i: abundance of the ith of S species observed; $n (= \sum_i x_i)$: total number of individuals in the sample; ω_{ij}: "distinctness weight" given to the path length linking species i and j in the taxonomy</p>	
Berger-Parker Index (Berger and Parker 1970)	$D = n_{max} / N$ <p>n_{max}: number of individuals of the most abundant species; N: total number of individuals</p>	<p>Low diversity 1 High diversity 0</p>
Margalef Index (Margalef 1969)	$D = (S - 1) / \ln(N)$ <p>S: number of species found; N: total number of individuals</p>	<p>Low diversity Low value</p>
Shannon-Wiener Index (Shannon and Weaver 1963)	$H' = - \sum p_i \log_2 p_i$ <p>p_i: proportion of abundance of species i in a community were species proportions are $p_1, p_2, p_3 \dots p_n$; S: number of species found</p>	<p>Low diversity 0 High diversity ≥ 5</p>
3. Ecological strategies		
Infaunal Trophic Index (Word 1979)	$ITI = 100 - 100/3 \left((n_1 + 1n_2 + 2n_3 + 3n_4) / (n_1 + n_2 + n_3 + n_4) \right)$ <p>Macrofauna groups: (1) suspension feeders; (2) interface feeders; (3) surface deposit feeders; (4) subsurface deposit feeders</p> <p>n_1, n_2, n_3, n_4: number of individuals in each of the above mentioned groups</p>	<p>Community dominated by: Group 1 >78 Group 2 58-77 Group 3 25-57 Group 4 < 24</p>
4. Thermodynamically oriented		
Eco-Exergy (Mejer and Jørgensen 1979)	$EX = T \times \sum \beta_i \times C_i$ <p>T: absolute temperature; C_i: concentration in the ecosystem of component i (e.g. biomass of a given taxonomic group or functional group); β_i: factor able to express roughly the quantity of information embedded in the genome of the organisms</p>	Higher value, higher biomass structure and information embedded in the biomass
Specific Eco-Exergy (Jørgensen 2000)	$SpEX = Exergy / Total \text{ biomass}$	Higher value, higher information embedded in the biomass

Table 2. Trophic classification of taxa contributing the top 80% of the abundance recorded in the two studied intertidal sites of the Mondego estuary, from 1985 to 2002. Trophic group assigned according to references. 1: suspension feeders; 2: suspension and surface-detritus feeders; 3: surface deposit feeders; 4: subsurface deposit feeders.

Trophic Group	Taxa	References	Trophic Group	Taxa	References
1	<i>Cerastoderma</i>	Morton 1983	3	<i>Carcinus maenas</i>	Riera et al. 1999
	<i>Spio</i>	Dauer et al. 1981		<i>Crangon crangon</i>	Ansell et al. 1999
	<i>Scrobicularia plana</i>	Riera et al. 1999		<i>Cyathura carinata</i>	Ferreira et al. 2004
2	<i>Alkmaria romijni</i>	Word 1990 (Ampharetinae)		<i>Hediste diversicolor</i>	Garcia-Arberas & Rallo 2002
	<i>Ampithoe</i>	Dixon & Moore 1997		<i>Hydrobia ulvae</i>	Philippart 1995
	<i>Corophium</i>	Word 1990		Diptera	Livingston 2003
	<i>Chaetozone setosa</i>	Word 1990		<i>Mysta picta</i>	Solis-Weiss et al. 2004
	<i>Heteromastus</i>	Word 1990	4	<i>Capitella</i>	Word 1990
	<i>Mediomastus</i>	Word 1990		Oligochaeta sp1	Word 1990
	<i>Melita palmata</i>	Word 1990		Tetrastemma	Barnes & de Villiers 2000
	<i>Pygospio elegans</i>	Word 1990		Tubificoides	Gaston et al. 1998
	<i>Streblospio shrubsolii</i>	Word 1990			

Table 3. Average abundance values (ind. m⁻²) of the species that contributed more than 70% to the difference between periods in the *Z. noltii* and in the bare sediment sites.

	Before the interruption	During the interruption	After the experimental opening
<i>Zostera noltii</i> site			
<i>Hediste diversicolor</i>	1189	19	523
Tubificidae	0	2944	714
<i>Alkmaria romijni</i>	204	993	443
<i>Heterosmastus filiformis</i>	265	550	357
<i>Capitela capitata</i>	114	241	92
<i>Chaetozone setosa</i>	0	1756	31
<i>Hydrobia ulvae</i>	1960	80691	41862
<i>Scrobicularia plana</i>	596	1023	724
<i>Melita palmata</i>	83	336	75
<i>Cyathura carinata</i>	271	217	173
Oligochaete	0	220	100
<i>Streblospio shrubsolii</i>	60	349	138
<i>Cerastoderma edule</i>	13	519	74
Bare sediment site			
<i>Hediste diversicolor</i>	523	50	265
<i>Hydrobia ulvae</i>	1166	27192	5573
<i>Alkmaria romijni</i>	93	6076	1483
<i>Cyathura carinata</i>	638	2043	2177
<i>Scrobicularia plana</i>	319	2347	2763
<i>Amphicteis gunneri</i>	106	0	0

Table 4. Summary of the ecological indicators trends obtained for the *Z. noltii* and bare sediment sites, considering “during the interruption” and “after the experimental opening” (after) periods. In bold are the values that are compliant with *a priori* established classification criteria.

Ecological indicator	Site	
	<i>Z. noltii</i>	Bare sediment
Margalef	↓ after	↓ after
Total Taxonomic Distinctness		
Taxonomic Distinctness	p>0.05	p>0.05
Eco-Exergy	↓ after	↑ after
Specific Eco-Exergy		
Berger-Parker	p>0.05	↓ after
Shannon-Wiener		↑ after
Taxonomic Diversity	p>0.05	↑ after
Infaunal Trophic Index		
AMBI	↓ after	p>0.05

Figures captions

Figure 1. Mondego estuary. Location of the two intertidal sampling sites (black circles): *Z. noltii* site and bare sediment site and position of the connection area between the North and South arms (open circle). NA-north arm, SA-south arm.

Figure 2. Temporal changes of *Z. noltii* meadows' area (ha) and biomass (g AFWD m⁻²), green macroalgae biomass (g AFWD m⁻²) and the connection width between the two estuarine arms. Black areas correspond to *Z. noltii* site, bare sediment site and connection between the two arms. NA-north arm, SA-south arm.

Figure 3. Macrofauna A. total abundance, B. total biomass and C. number of species in the *Z. noltii* site and in the bare sediment site, during the study period.

Figure 4. A Two-dimensional, non-metric, multidimensional scaling (nMDS) ordination based on abundance data. A. *Z. noltii* site and B. bare sediment site, for the three time periods: before the perturbation under assessment (1985 and 1986), period without connection between arms (1993, 1994 and 1995) and after experimental mitigation measures (1999-2002). Each point represents a different time period at the same site. Stress values are shown for each nMDS plot indicating the similarity between the multi-dimensional representation and the 2-dimension plot.

Figure 5. Temporal and spatial variation of different ecological indicators applied to data of the intertidal communities of the South arm of the Mondego estuary, during the study period.

Figure

Figure

Figure 3

A. *Zostera noltii* site **B. Bare sediment site**

● Before perturbation ▼ During interruption □ After experimental opening

A. Margalef **B. Total Taxonomic Distinctness, $s\Delta^+$** **C. Taxonomic Distinctness, Δ^*** **D. Taxonomic Diversity, Δ** **E. Infaunal Trophic Index** **F. Shannon-Wiener** **G. Berger-Parker** **H. AMBI** ● *Zostera noltii* site

● Bare sediment site

Figure 5

I. Eco-Exergy ($\text{g m}^{-2}\text{det.energy.equiv.}$)

J. Specific Eco-Exergy (Eco-Exergy/biomass unit)

Figure 5 (continued)