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Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal)

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Abstract

The need for new tools to assess the environmental status of coastal and estuarine systems encouraged Borja et al. [Mar. Pollut. Bull. 40 (2000) 1100] to develop a new index, the AZTI's Marine Biotic Index (AMBI), which needs to be tested as much as possible in different geographical areas to assess its applicability. This index was applied in the Mondego estuary (western coast of Portugal) together with the Shannon–Wiener, Margalef, Simpson, and *W*-statistic indices, which are widely used in detecting the effects of marine pollution. Results show that, in some cases, the AMBI provides a more accurate assessment of environmental conditions than the other indices, which were influenced by the dominance of certain species, allowing us to consider it as a promising tool to characterize marine and estuarine environmental quality.

Keywords: Benthos; Environmental quality; Estuaries; Marine biotic index; Soft-bottom; Water Framework Directive

1. Introduction

There is an increasing need for techniques capable of evaluating changes in the coastal and estuarine environments. Application of the Water Framework Directive (2000/60/EC) in European Union (EU) countries has required the development of new biological indices capable of distinguishing different levels of ecological quality and classifying coastal areas as very good, good, moderate, poor or bad.

It was for this reason that Borja et al. (2000) developed the AZTI's Marine Biotic Index (AMBI) that assesses the response of soft-bottom communities to natural and man-induced changes to the environment, integrating long-term environmental conditions.

For the development of the AMBI, the soft-bottom macrofauna is divided into five groups according to their sensitivity to an increasing stress:

(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. These species may occur under normal conditions, but their populations are stimulated by organic enrichment;
(IV) Second-order opportunist species, mainly small sized polychaetes; or

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(V) First-order opportunist species, essentially deposit-feeders.

For the application of this index, nearly 2000 taxa representative of the most important soft-bottom communities present in European estuarine and coastal systems have been classified.

Many authors have used indicator species as a tool for evaluating marine systems. Limnological studies were pioneer in using indicator species to determine the quality of the aquatic environment. For instance, the saprobies system (Kolkwitz and Marsson, 1902, 1908, 1909) constitutes the first approach to establish a biological index able to show different status of deterioration and progressive recuperation of organisms and communities as a response to the effects of organic enrichment in the waters. The presence of a species or a group of species is one of the most commonly used parameters to detect marine pollution, especially organic pollution (Pearson and Rosenberg, 1978). Authors such as Warwick (1993) consider that the use of indicator species is only applicable to organic pollution studies, including in some cases, oil pollution. Even so, there is no sound methodology to establish the level to which a certain community must be dominated by a particular indicator species to confirm that it is affected by any given type of perturbation. This implies an important exercise of subjectivity and the impossibility of setting up fixed reference values.

Nevertheless, the design of the AMBI might allow establishing a set of values to achieve a system classification. One of the requirements for an index to be a useful ecological indicator is that it should be applicable in any geographical area. So far, the AMBI has been tested in a restricted number of cases along the European coast (Borja et al., 2000, 2003a, 2004), so it should be used more often in other systems to test its applicability. Studies carried out in the Mondego estuary (Portugal), a eutrophic system, during the past decade provided a large data base, and therefore, it can be considered an excellent test of the AMBI in a new system. Additionally, the comparison between the AMBI and other more traditional and widely used indices, such as the Shannon–Wiener, Margalef, or Simpson indices as well as the ABC method (by means of *W*-statistic) can evaluate in what extent the use of the AMBI might be advantageous in detecting environmental disturbance in coastal systems. Other indices such as the environmental condition index (Engle et al., 1994) or the Chesapeake Bay B-IBI index (Weisberg et al., 1997), which combine diversity measures, taxonomic, and trophic composition due to the fact that they were designed for very specific geographic areas in North America, were not taken into account in this study.

2. Materials and methods

2.1. Study site and data sets

The study site was the Mondego estuary in the western coast of Portugal. This system is under severe environmental stress, and an ongoing eutrophication process has been monitored during the last decade. Details about the system can be found in available literature (e.g. Marques et al., 1993a,b, 1997; Flindt et al., 1997; Lopes et al., 2000; Pardal et al., 2000; Martins et al., 2001; Cardoso et al., 2002).

Two different data sets were chosen to estimate the different indices. The first data set was provided by study at 14 stations in 1998 and 2000 (Fig. 1) describing the subtidal soft-bottom communities, throughout the whole system with regard to species composition and abundance, taking into account spatial distribution in relation to the physicochemical factors of water and sediments. The second selected data set was produced by a study (February 1993–February 1994) on the intertidal benthic communities in the south arm of the estuary (Fig. 2). Samples of rooted macrophytes, macroalgae, and associated macrofauna as well as samples of water and sediments were taken fortnightly at different sites, during low water, along a spatial gradient of eutrophication symptoms. The gradient ranged from a non-eutrophic zone (where a rooted macrophyte community of *Zostera noltii* is present), to a heavily eutrophic zone, in the inner areas of the estuary (where the rooted macrophytes disappeared and green macro algal blooms of *Enteromorpha* spp. occurred during the last decade). In this area, as a pattern, *Enteromorpha* spp. biomass normally increases from mid winter (February/March) to July, when an algal crash usually occurs. A second, but much less important, macro algal peak may sometimes be observed in September, followed by a biomass decrease up to the winter (Marques et al., 1997).

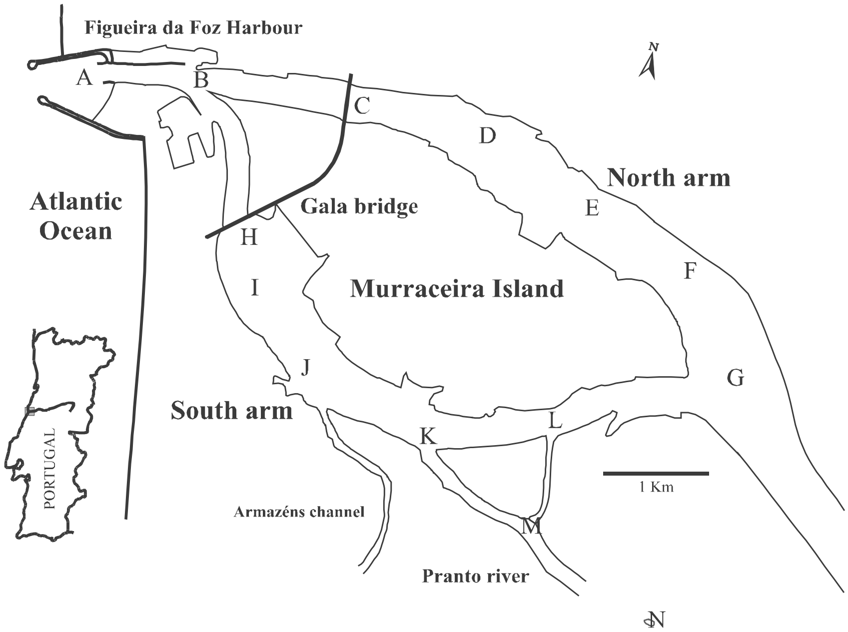

Fig. 1. The Mondego estuary. Location of the subtidal sampling stations.

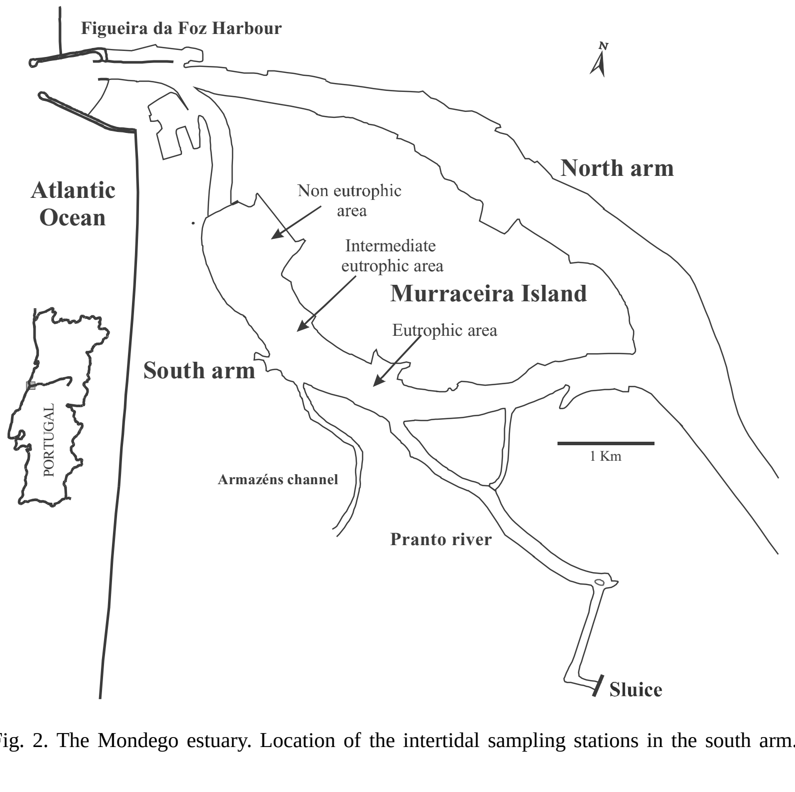

Fig. 2. The Mondego estuary. Location of the intertidal sampling stations in the south arm.

In both studies, organisms were identified to the species level and their biomass was determined (g m^{-2} AFDW). Corresponding to each biological sample, the following environmental factors were also measured: salinity, temperature, pH, dissolved oxygen, silica, chlorophyll *a*, ammonia, nitrates, nitrites, and phosphates in water, and organic matter content in sediments.

2.2. Data analysis

2.2.1. Indices application

Besides the AMBI (Borja et al., 2000), the following indices were applied: Shannon–Wiener, Margalef, Simpson (e.g. Magurran, 1988), and the *W*-statistic (Clarke, 1990), which is based on the ABC method (Warwick, 1986). The marine biotic index was applied using the AMBI© software (Borja et al., 2003a and http://www.azti.es, where the software is freely available).

An increase in the values of the Shannon–Wiener and Margalef indices is usually understood as an improvement in the state of the system, while it means exactly the contrary in the case of AMBI and the Simpson index.

2.2.2. Data treatment

The chosen indices estimation was based on each data set aiming to differentiate between sampling areas along the spatial gradient, and a one-way ANOVA was applied to test if differences observed were statistically significant. The purpose was to compare the performance of the different indices in distinguishing between different well-known environmental scenarios. Moreover, Pearson's correlations were applied to analyse the response of each index as a function of different environmental variables, and to identify any significant parallelisms between the patterns of variation of different indices.

3. Results

The data set from the subtidal communities of both arms of the estuary showed that the indices estimated for 1998 and 2000 were significantly correlated ($P \leq 0.05$), suggesting a similar evaluation of the system (Table 1). None of the computed indices showed any significant relation with the physicochemical environmental variables. Some inconsistencies between the assessments provided by the AMBI and the other indices are described below:

Table 1
Pearson correlations between the values of the different indices estimated in 1998 and 2000 at the 14 sampling stations located in the two arms of the Mondego

	AMBI	Shannon–Wiener	Margalef	Simpson
Shannon–Wiener	−0.73**			
Margalef	−0.69*	+0.83**		
Simpson	+0.74**	−0.98**	−0.84**	
W-statistic	−0.45*	+0.75**	+0.72*	−0.81**

* $P = 0.05$.
** $P = 0.01$.

(a) While the diversity indices and the *W*-statistic show a worsening of the system at station A between 1998 and 2000 (Table 2), the AMBI suggests, to the contrary, an improvement. In fact, in 1998 the AMBI reveals co-dominance among species of the group I (54.2%), group II (10.8%), and group III (35.0%); while in 2000, only group I (51.3%) and group II (48.7%) were represented. The decrease in environmental quality described by the other indices is basically due to the dominance of *Elminius* spp. in station A during 2000. Although this species does not indicate any kind of pollution, its abundance caused a decrease in diversity values as the Shannon–Wiener and Simpson indices depend on species richness and evenness. Also, the *W*-statistic was influenced by the dominance of *Elminius* spp. because by coincidence, these species are very small in size. AMBI indicated a reduction of pollution tolerant species (group III) and an increase in species indifferent to organic enrichment (group II).

(b) According to the values of the diversity indices and *W*-statistic, for stations B and C, the environmental quality of the systems should be improving (Table 2), while AMBI shows a worsening. For station B, the decline occurs drastically (from 1.98 in 1998 to 3.5 in 2000), changing from what could be considered an unbalanced community, in which species belonging to ecological group I prevailed (42.9%) to a transitional pollution state

Table 2
Values of the different indices estimated at the 14 sampling stations in the Mondego estuary (campaigns from 1998 to 2000)

Station	Shannon–Wiener		Margalef		Simpson		*W*-statistic		AMBI	
	1998	2000	1998	2000	1998	2000	1998	2000	1998	2000
A	2.64	0.90	2.32	1.44	0.23	0.76	0.27	−0.19	1.21	0.73
B	2.45	3.44	1.08	4.01	0.18	0.14	0.40	0.20	1.90	3.50
C	1.36	2.40	0.89	1.52	0.50	0.24	0.21	0.23	3.10	3.90
D	2.77	1.84	1.99	0.89	0.15	0.28	0.59	0.39	2.70	2.30
E	2.14	0.65	1.26	0.27	0.26	0.71	0.30	−0.50	1.70	2.40
F	2.61	1.37	1.55	0.66	0.21	0.41	−0.05	0.20	1.60	0.75
G	0.87	2.03	0.60	1.23	0.67	0.33	0.18	0.19	3.00	3.00
H	0.00	2.55	0.00	1.73	1.00	0.18	−1.00	0.45	7.00	2.30
I	1.43	2.92	0.94	1.99	0.55	0.11	−0.15	0.50	2.00	2.60
J	2.03	2.51	1.07	1.34	0.39	0.22	−0.06	0.24	3.13	3.00
K	1.91	1.46	1.25	1.02	0.35	0.51	0.22	0.06	2.02	2.90
L	1.66	2.39	0.81	1.43	0.42	0.25	−0.04	0.11	3.00	3.00
M	1.32	1.68	0.98	1.14	0.52	0.38	−0.20	−0.09	2.94	2.80
N	0.63	1.38	0.72	0.79	0.74	0.51	−0.18	0.24	3.00	3.00

revealed by the dominance of species of ecological group III (43.8%) and group IV (41.6%). Station C changed also to a transitional pollution state or even moderately polluted state (AMBI: 3.9) as a function of the dominance of ecological group III (48.8%), group IV (41.5%), and group V (9.7%).

Assuming that the environmental state in stations B and C was accurately assessed by the AMBI as moderately polluted, the observed increase in diversity may result from the co-existence of multiple species from polluted and unpolluted sites, which often occurs in moderately disturbed areas. Results from *W*-statistic, which is, in principle, efficient in distinguishing moderate disturbance situations (i.e. organic enrichment), are harder to interpret. Stations B and C (B: $W = +0.20$; C: $W = +0.23$), based on the *W*-statistic, should be considered unpolluted. One explanation for this interpretation would be that the environmental stress is not sufficient to reduce the size of macrofaunal individuals such that it causes the curves of abundance and biomass to cross.

By applying a one-way ANOVA to 1998 results (Table 3), it can be verified that the indices, except the AMBI, were efficient in distinguishing between stations from the north and south arms of the estuary: values estimated in the south arm consistently indicated a higher disturbance. Although values estimated for AMBI in the south arm stations were also higher indicating a degrading situation, the differences were not statistically significant. On the other hand, with regard to the year 2000 results, none of the indices utilised revealed significant differences between the stations of both arms of the estuary (Table 4).

In spite of their different behaviours, except the AMBI, all indices were able to differentiate the three sampling areas along the south arm (Table 5). However, contrary to expectations, the Shannon–Wiener

Table 3
Values obtained after the application of a one-way ANOVA test considering the sampling stations located in the two arms of the Mondego estuary in 1998

Arm	*n*	Mean	*F*	*P*
Shannon–Wiener				
North	6	2.32	10.47	0.007
South	8	1.23		
Margalef				
North	6	1.51	8.40	0.013
South	8	0.79		
Simpson				
North	6	0.25	10.68	0.006
South	8	0.58		
W-statistic				
North	6	0.28	6.53	0.025
South	8	−0.15		
AMBI				
North	6	2.03	2.65	0.13
South	8	3.38		

Table 4
Values obtained after the application of a one-way ANOVA test considering the sampling stations located in the two arms of the Mondego estuary in 2000

Arm	*n*	Mean	*F*	*P*
Shannon–Wiener				
North	6	1.76	0.65	0.43
South	8	2.15		
Margalef				
North	6	1.46	0.07	0.79
South	8	1.33		
Simpson				
North	6	0.42	1.06	0.32
South	8	0.31		
W-statistic				
North	6	0.05	1.23	0.28
South	8	0.21		
AMBI				
North	6	2.26	1.39	0.26
South	8	2.82		

Table 5
Values obtained after the application of one-way ANOVA test considering the three sampling areas located along the spatial gradient of eutrophication symptoms in the south arm of the Mondego estuary in 1993–1994

Area	*n*	Mean	*F*	*P*
Shannon–Wiener				
Non-eutrophic	35	0.78	17.12	0.00003
Eutrophic	35	1.69		
Intermediate eutrophic	35	1.14		
Margalef				
Non-eutrophic	35	2.17	13.78	0.00004
Eutrophic	35	1.52		
Intermediate eutrophic	35	1.86		
Simpson				
Non-eutrophic	35	0.79	21.21	0.00001
Eutrophic	35	0.48		
Intermediate eutrophic	35	0.68		
W-statistic				
Non-eutrophic	35	−0.01	6.27	0.002
Eutrophic	35	0.04		
Intermediate eutrophic	35	−0.02		
AMBI				
Non-eutrophic	35	3.07	3.36	0.06
Eutrophic	35	3.07		
Intermediate eutrophic	35	3.23		

Table 6
Pearson correlations between the values of the different indices estimated in 1993/1994 and the nutrient concentrations at the three sampling areas along the spatial gradient of eutrophication symptoms in the south arm of the Mondego estuary

	$[NO_3^-]$	$[NO_2^-]$	$[NH_4^+]$	$[PO_4^-]$
Shannon–Wiener	+0.55*	+0.51*	+0.43*	+0.16
Margalef	+0.23	+0.17	+0.21	+0.58*
Simpson	+0.64*	+0.52*	+0.47*	−0.18
W-statistics	+0.48*	+0.50*	+0.42*	−0.22
AMBI	+0.18	−0.07	+0.15	−0.35*

* $P \leq 0.05$.

and Simpson indices as well as the *W*-statistic showed higher values in the most heavily eutrophic zone (1.69; 0.48; and 0.04, respectively) than in the *Z. noltii* meadows (0.78; 0.79; −0.01, respectively). As expected, the Margalef index exhibited higher values at the *Z. noltii* beds and lower values in the inner areas of the south arm.

As shown by Pearson correlations (Table 6), the Shannon–Wiener and Simpson indices as well as the *W*-statistic showed significant positive correlations with ammonium, nitrite, and nitrate concentrations in the water column along this gradient, while the Margalef index and the AMBI were significantly correlated with phosphate concentration levels ($r = +0.58$, $P = 0.05$; $r = -0.34$, $P = 0.05$).

In all three areas, estimated values of the AMBI were close to 3, which indicate slightly polluted scenarios, sensu Borja et al. (2000), where species of the ecological group III dominate. However, AMBI values between 4 and 5 were estimated from 22 July to 1 October in intermediate eutrophic zone (Fig. 4B), which indicates a moderately polluted situation.

4. Discussion and conclusions

In general, from the results of this study, the AMBI index worked satisfactorily. It is true that in some cases responses and performances were different from other indices, but such unlike responses appear to be due to system-specific causes. For instance, depending on the indices applied (e.g. Shannon–Wiener, Simpson), the dominance, for unclear reasons, of certain species in given areas of the Mondego estuary produced low diversity estimates, although those species belonged to ecological groups usually related to non-polluted environments. Besides diversity indices, some inconsistencies were also found between the AMBI and *W*-statistic responses. Again, results from *W*-statistic were confusing due to the strong dominance of species that are non-pollution indicators (e.g. *Hydrobia ulvae* and *Cerastoderma edule*) (Warwick and Clarke, 1994). A similar situation was observed in a study performed by Beukema (1988), where the dominance of *Corophium volutator* (Amphipoda) and *H. ulvae* caused the use of ABC method, in which the *W*-statistic is based to indicate non-polluted areas as disturbed.

On the other hand, the AMBI was inefficient in discriminating among areas with clearly different eutrophication symptoms along the spatial gradient in the south arm of the estuary (e.g. dominance of *Z. noltii* or *Enteromorpha* spp. as main primary producers). This may perhaps be explained if we consider that eutrophication effects at the primary producer level, which are clearly visible, are still not as evident at the other benthic trophic levels (Marques et al., 2003). In fact, although a number of shifts in species composition are already recognizable, the benthic community structure in the three zones along the spatial gradient shows, to a great extent, the same dominant groups (Marques et al., 2003). With other impact sources, such as outfalls, oil platforms, etc. it has been demonstrated that AMBI shows clearly the stress gradient (Borja et al., 2003a; Muxika et al., 2003). The AMBI values estimated in the Mondego estuary were similar at the three sampling areas due to the common dominance of *H. ulvae,* which belongs to the ecological group III. The remainder of the indices are strongly affected by the large abundance of *H. ulvae* and *C. edule*, the dominant species, although such dominance does not have anything to do with eutrophication symptoms, being rather related to more food resources availability (Pardal et al., 2000).

Unlike other indices (Figs. 3A–C and 4A), AMBI does not vary with time (Fig. 4B), since it is not influenced by changes in species abundance. This is important because during the period of study, there were no changes in pollution condition. AMBI appears, nevertheless, to be efficient in detecting changes related to macroalgal dynamics. The increase in the AMBI values resulted from a strong dominance of polychaetes, *Capitella capitata* (888 indv m^{-2}) and *Chaetozone setosa* (30,179 indv m^{-2}). These two species have been widely mentioned in the literature as indicators of organic pollution (i.e. Bellan, 1967, 1984; Glémarec and Hily, 1981). Pardal (1998) observed that a strong increase in polychaetes abundance at the intermediate eutrophic zone occurred after heavy algal mortality events (algal "crash") in the innermost areas of the south arm. However, the two observations might not be directly related. Lopes et al. (2000) observed an increase on the green macroalgal biomass in the intermediate eutrophic zone during the same period, which appeared to be clearly related with the substantial increase in the abundance of *C. capitata*. Thus, although the growth of polychaetes populations in the intermediate eutrophic zone appears to be related with algal dynamics, it is not clear if there is any relation between the augmentation of algal density in this zone and the algal crash in the inner areas of the estuary.

One of the most obvious advantages of using the AMBI is that it provides a classification of the system that matches satisfactorily the one established by the European Water Framework Directive (WFD). According to the WFD, the biological quality elements regarding benthic communities must account not only for indicator species but also diversity and evenness. A tentative correspondence between the categories established based on the AMBI and the ones from the WFD could be the following:

AMBI	WFD (status)
Unpolluted	High
Slightly polluted	Good
Moderately polluted	Moderate
Heavily polluted	Poor
Extremely polluted	Bad

but some advanced discussion about this particular subject can be consulted in Borja et al. (2003b, 2004).

Such correspondence cannot be established using any of other tested diversity indices. In other words, diversity indices allow the comparison of different areas in terms of their diversity, but these indices cannot classify a system regarding the environmental quality. In general, low estimates of diversity indices (e.g. Shannon–Wiener) are considered as an indication of environmental stress (e.g. pollution) (Anger,

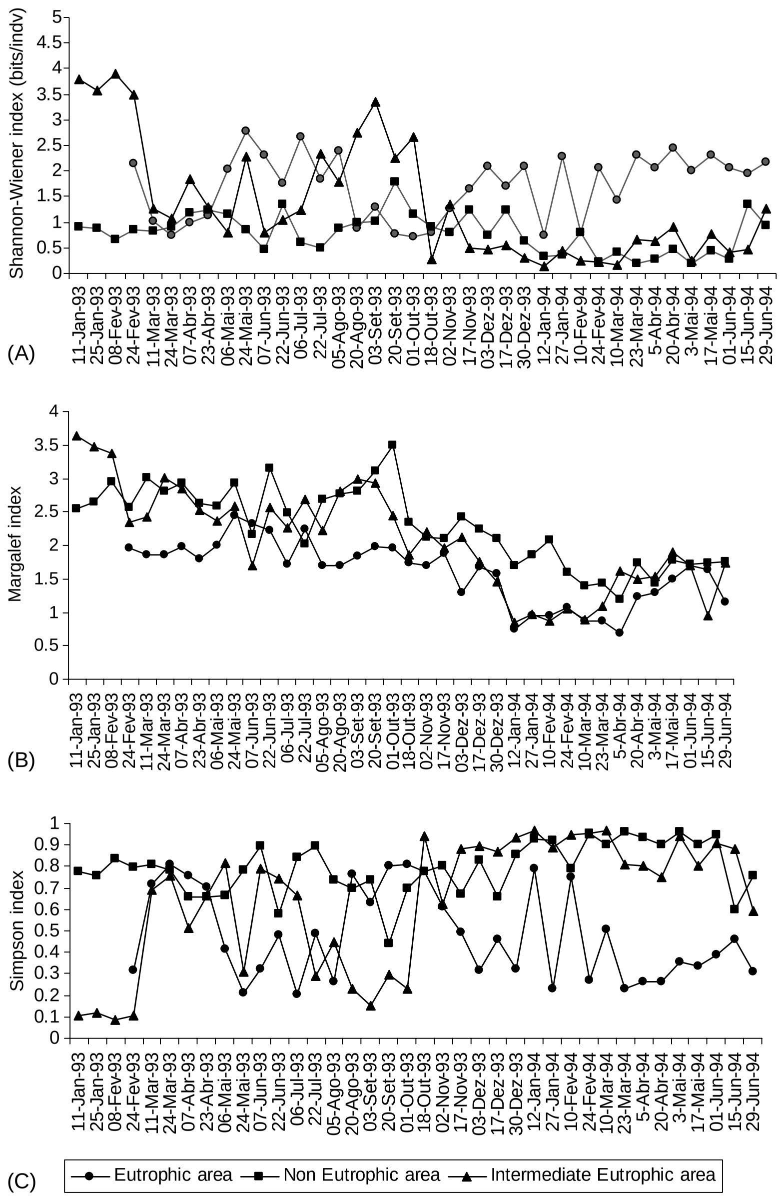

Fig. 3. Temporal and spatial variation of the Shannon–Wiener index (A), Margalef index (B), and Simpson index (C) in the south arm of Mondego estuary.

1975; Pocklington et al., 1994; Engle et al., 1994; Yokoyama, 1997). One of the practical problems when using them is the lack of objectivity in setting the threshold that indicates the effects of such stress. Detractors of diversity indices based their criticisms on the lack of accuracy in detecting initial states of pollution (Leppäkoski, 1975; Pearson and Rosenberg, 1978; Rygg, 1985). In our case study, we verified that even in the case of *Z. noltii* beds, where the benthic community is supposed to be better structured than in zones depending on the dynamics of green macroalgae (*Enteromorpha* spp.), the diversity values were low.

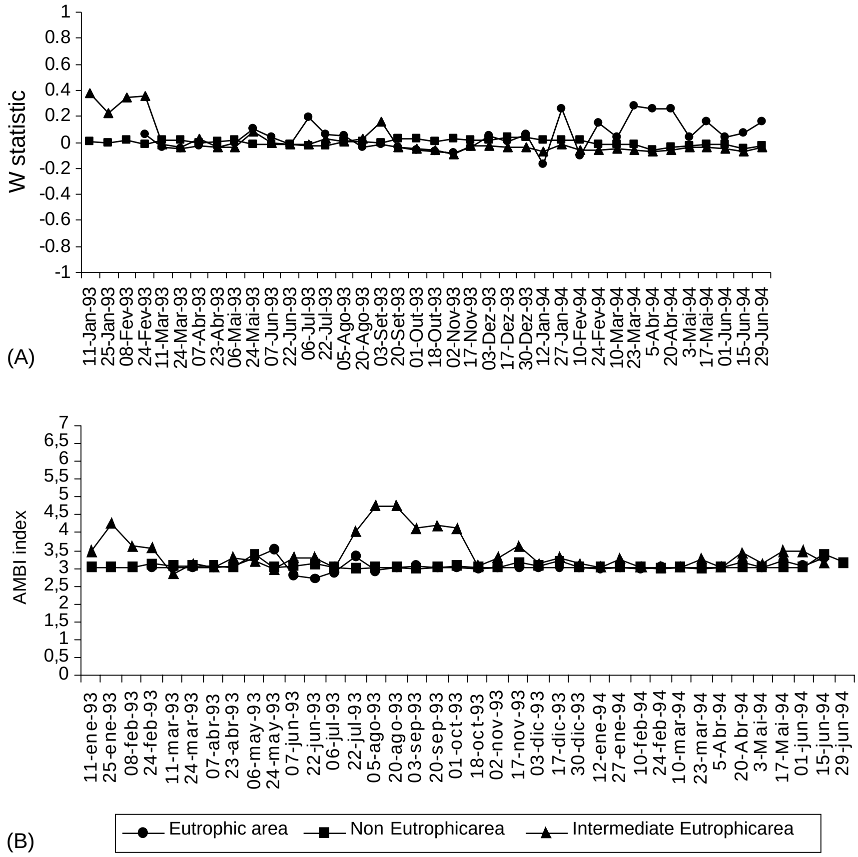

Fig. 4. Temporal and spatial variation of the *W*-statistic (A) and AMBI index (B) in the south arm of Mondego estuary.

The *W*-statistic is capable of distinguishing between non-disturbed, slightly disturbed, and disturbed situations and does not depend on reference values. Nevertheless, the not unusual dominance of certain species small in size and characteristic of non-polluted environments will lead to erroneous evaluations, as illustrated by several case studies (Ibanez and Dauin, 1988; Beukema, 1988; Weston, 1990; Craeymeersch, 1991).

Experience demonstrates that none of the available measures regarding biological effects of pollution should be considered ideal. The classification of species as indicators of different degrees of pollution, which constitutes the base of the AMBI, often contains subjective elements; in fact, the interpretation regarding the meaning of the presence of a given species may be ambiguous. For instance, *C. setosa*, depending on the authors, is considered indicator of moderate pollution (Bellan, 1967; Solís-Weiss, 1982) or of intense pollution (Glémarec and Hily, 1981; Glémarec et al., 1982; Majeed, 1987). Also, *Spiochaetopterus costarum* is considered by Bellan (1967) as an indicator of slightly polluted environments and by López Jamar (1985) as characteristic of highly polluted areas. Similarly, *Nereis caudata* is considered indicator of intense pollution by Bellan (1967), Zabala et al. (1983), and Lardicci et al. (1993) and simply tolerant by Glémarec and Hily (1981), Glémarec et al. (1982), and Majeed (1987).

As a general conclusion, the complementary use of different indices or methods based on different ecological principles is highly recommended in determining the environmental quality of a system, as already stated by Dauer et al. (1993). Additionally, until now, results of case studies in which the AMBI were applied have been very satisfactory, and therefore, it appears to be a promising tool for assessing environmental quality in coastal marine systems. One must obviously take into account that an index such as AMBI, which is based on information on species response to pollution, is under permanent actualisation in terms of database. This can only be achieved through studies that contribute to improve the existing species classification and through application in as many situations as possible.

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