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User-friendly guide for using benthic ecological indicators in coastal and marine quality assessment

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Abstract

Experience demonstrates that none of the available measures on biological effects of pollution should be considered ideal. The use of a single approach does not seem appropriate due to the complexity inherent in assessing the environmental quality of a system. Rather, this should be evaluated by combining a suite of indices providing complementary information.

Having this in mind, a key table is proposed in this work with the aim of helping managers and authorities of coastal areas in selecting the most suitable ecological indicators taking into account the type of disturbance and the data available. Such key includes numerous indices based on benthic invertebrate fauna information, because in the case of coastal and transitional waters ecosystems there is a clear preference for benthic communities, which integrate environmental conditions and changes in an a very effective way if we want to monitor long-term responses and site-specific impacts.

The development of this key was based not only on theoretical approaches, but also on results from its application using data bases corresponding to different geographical areas (the Mondego estuary, in the North-Western Coast of Portugal, and Mar Menor coastal lagoon, Escombreras basin, and Cabo Tiñoso in the South-Eastern coast of Spain).

Some recommendations are provided with regard to the most adequate application of the indices, as for example, in what situations it is not advisable the use of some of them, depending on the type of disturbance or the level of taxonomic identification of the organisms.

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1. Introduction

1.1. What are indicators and what is their utility?

Ecological indicators are commonly used to supply synoptic information about the state of ecosystems. Most often they address ecosystem's structure and/or functioning accounting for a certain aspect or component, for instance nutrient concentrations, water flows, macro-invertebrates and/or vertebrates diversity, plants diversity, plants productivity, erosion symptoms and, sometimes, ecological integrity at the system's level.

Indicators are quantitative representations of the forces that drive a system, of responses to forcing functions, or of previous, current, or future states of a system. When they are used effectively, indicators are expected to reveal conditions and trends that help in development planning and decision-making.

The main attribute of an ecological indicator is to combine numerous environmental factors in a single value, which might be useful in terms of management and for making ecological concepts compliant with the general public understanding. Moreover, ecological indicators may help in establishing a useful connection between empirical research and modelling, since some of them are of use as orientors (also referred in the literature as goal functions) in ecological models [1].

In general, applications of ecological indicators are not exempt of criticisms. The first is that the aggregation results in oversimplification of the ecosystem under observation. Moreover, problems arise from the fact that indicators account not only for numerous specific system characteristics, but also other kind of factors (e.g. physical, biological, ecological, socio-economic, etc). Thus, indicators must be utilised following the right criteria and in situations that are consistent with its intended use and scope; otherwise they may lead to confusing data interpretations.

1.2. Use of indicators: what is a good indicator? what are the characteristics of a good indicator?

Waltz (2000) and Meadows (1998) in Unesco [2] provided the following series of characteristics for environmental indicators: (a) to have an agreed, scientifically sound meaning, (b) to be representative of an important environmental aspect for the society, (c) to provide valuable information with a readily understandable meaning, (d) to be meaningful to external audiences, (e) to help focusing information to answer important questions, and (f) to assist decision-making by being efficient and cost-effective to use.

From a more ecological point of view, we may say that the characteristics defining a good ecological indicator are (a) handling easiness, (b) sensibility to small variations of environmental stress, (c) independence of reference or control samples, (d) applicability in extensive geographical areas and in the greatest possible number of communities or ecological environments and (e) relevance to policy and management needs.

It is not easy to fulfil all these requirements and, in fact, despite the panoply of bio-indicators and ecological indicators that can be found in the literature, very often they are more or less specific for a given kind or stress or applicable to a particular type of community and/or scale of observation, and rarely their validity have in fact been utterly proved. On the other hand, more and more became difficult to find reference sites or controls showing really natural communities to compare with human-affected localities.

In this sense, the independence of one indicator means that its range of values and its behaviour with stress are well known (as in the case of body temperature in human health). However, this criterion is nearly a utopia considering the low level of knowledge of the community responses to changes in the environmental conditions in the marine environment.

Applied and focused on different ecosystem compartments in the marine environment, bioindicators can be found in pelagic communities as well as in benthic ones, being specific or belonging to vegetal and faunal communities.

Pelagic communities are useful in detecting changes at large scale, but their high spatial heterogeneity and the water masses mobility invalidate them when detecting local effects. Besides, the high changing rates in plankton communities makes its response to be nearly simultaneous with environmental alterations. Actually, this is the reason why they are not useful as historical registers of sporadic pollution episodes, and are only relevant during or immediately after the disturbance occurrence. Due to that, benthic communities are usually considered more adequate than those of the pelagic domain to evaluate the status of an aquatic ecosystem. In fact, because of their limited mobility, benthic organisms are more sensitive to local disturbance, and as a result of their permanence over seasons; they integrate the recent history of disturbances that might not be detected in the water column.

Regarding the benthic communities, if asked about the suitability of using macrobenthos or meiobenthos, in our opinion, both options are valid. Whereas the response of macrobenthos to different disturbances, with the replacement of a given community for another that reflects the new environmental conditions, normally takes years, the meiobenthos response is much faster and can be stated in months [3]. However, the taxonomical difficulty concerning the identification of meiobenthic organisms made the macrobenthos the most commonly used component of the marine system in environmental impact studies.

1.3. Review of benthic ecological indicators and their specificities

In the last years, the increasing need for stable and comparable criteria of environmental quality in the European aquatic ecosystems (including coastal zones and estuaries), which follow the promulgation of the European Union Water Framework Directive, reactivated at the end of 2000 the use and search of pollution biological indicators.

Many ecological indicators used and/or tested in evaluating the status of these ecosystems are found in the literature and some authors (e.g. Blandin [4]; Rice [5]) developed bibliographical reviews about the use of such indicators in environmental studies.

Some ecological indices are focused on the presence/absence of a given indicator species, while others take into account the different ecological strategies adopted by organisms, the diversity, or the energy variation in the system through changes in the biomass of individuals. Other group is thermodynamically oriented or it is based on network analysis, including indices that capture the ecosystem information from a more holistic perspective. A last group tries to include all the information about the environment in one single value through the so-named integrity indices.

1.3.1. Indices based on indicator species

When talking about indicator species, we must distinguish two situations that can lead to some confusion, as we can refer to the use of ''in situ'' or experimental measurements to quantify the toxic pollution effects.

When talking about indicator species in nature, we refer to those ones which their occurrence and dominance is associated to an environmental deterioration. They may be favoured for such fact or for their tolerance to that type of pollution in comparison to other less resistant species. In a sense, the possibility of assigning a certain grade of pollution to an area in terms of the present species has been pointed out by a number of researchers as Reish [6], Bellan [7] or Glemarec et al. [8], mainly in organic pollution studies.

Indices based on faunal communities, such as the Bellan's one (based on polychaetes) or the Bellan–Santini's one (based on amphipods), attempt to characterise environmental conditions by analysing the dominance of species indicating some type of pollution in relation to species considered as indicative of an optimal environmental situation [9,10]. Several authors considered as not advisable the use of these indices because often such indicator species may occur naturally in relative high densities. The point is that no reliable methodology exists to know at which level one of those indicator species can be well represented in an unaffected community, leading to a significant exercise of subjectivity [11]. Despite these criticisms, even recently, the AMBI Index [12], based on the Glemarec and Hily [13] species classification regarding pollution, as well as the BENTIX proposed by Simboura and Zenetos [14], or the Indicators Species Index (ISI) [15], all of them applying the same principles, have gone back to update such pollution detecting tools. Moreover, Roberts et al. [16] also proposed an index based on macrofaunal species which accounts for the ratio of each species abundance in the control vs. in samples proceeding from stressed areas. It is however a semi-quantitative index as well as site and pollution type specific. In the same way, the Benthic Response Index [17] is based upon the type of species (pollution tolerance) in a sample, but its applicability is complex as it is calculated by using a two-step process in which ordination analysis is employed to quantify a pollution gradient within a calibration data set.

Regarding submerged vegetation, there are a number of Genera that universally appear when pollution situations occur. Among them, there are the green macroalgae as *Ulva, Cladophora* or *Chaetomorpha* and red macroalgae as *Gracilaria, Porphyra* and *Corallina*.

High structural complexity species, such as Phaeophyta belonging to Fucales and Laminariales orders, are seen worldwide as the most sensitive to any kind of pollution, with the exception of such species of *Fucus* genus that cope with moderate pollution [18]. On the other hand, marine Spermatophytae are considered indicator species of good quality in the water.

In the Mediterranean Sea, for instance, the presence of *Cystoseira* and *Sargassum* or *Posidonia oceanica* meadows indicates good water quality. According to some authors [19], monitoring the population density and distribution of such species allows the detection and evaluation of the impact of whatever activity. In fact, *Posidonia oceanica* is possibly the mostly used water quality indicator of in the Mediterranean Sea [20], as well as the Conservation Index [21], which is based on the named seagrass.

Orfanidis et al. [22] introduced a new Ecological Evaluation Index (EEI) for the evaluation of ecological status of transitional and coastal waters in accordance to the European Water Framework Directive. This index is based on the classification of the marine benthic macrophytes in two ecological state groups (ESGs I, II) representing alternative ecological states (pristine and degraded).

Besides that, there are species able to resist and to accumulate diverse pollutant substances in their tissues better than others. These species facilitate the detection of such pollutants even when their low environmental levels turn difficult their detection by usual analytical techniques [23].

Molluscs, namely bivalves, have been one of the most used to determine a toxic substance existence and quantity its concentration. Individuals of the genus *Mytilus* [24], *Cerastoderma* [25], *Ostrea* [26] and *Donax* [27] have been considered ideal in many works to detect the concentration of a toxic substance in the environment, namely due to their sessile nature, wide geographical distribution and capability to detoxify when pollution ceases. Likewise, amphipods have been considered capable to accumulate toxic substances [28], as well as species of polychaetes such as *Nereis diversicolor* [29], *Neanthes arenaceodentata* [30], *Glycera alba*, *Tharix marioni* [31] or *Nephtys hombergi* [32]. Other authors (e.g. Newman et al. [33]; Storelli and Marcotrigiano [34]) considered algae as optimal for detecting heavy metals, pesticides and radionuclides, being *Fucus*, *Ascophyllum* and *Ulva* the most utilised.

For the sake of comparison, the substances concentrations in organisms must be translated to uniform and comparable units. This is done through the Ecologic Reference Index (ERI), which represents a potential for environmental effects. This index has only been applied using blue mussels [35].

A few indices based on the use of in situ-data measurements were formulated. The simple measurement of pollutants effects (e.g., % incidence, % mortality) on those species or the use of biomarkers (based on species that are sensitive enough to certain contaminants) useful to evaluate the specificity of their rapid responses to natural or anthropogenic changes have been the commonest approaches. In this sense, in 2002, the Working Group on Biological Effects of Contaminants (WGBEC) recommended different techniques for biological monitoring programmes. Despite this last possibility, it is difficult for the environmental manager to interpret increasing or decreasing changes in biomarkers' data. Nevertheless, the use of in situ data and laboratory measurements may be used in complementary ways to assess and report on ecosystem and species health.

1.3.2. Indices based on ecological strategies

Some indices try to assess environmental stress effects accounting for the ecological strategies followed by different organisms. This is the case of trophic indices such as the Infaunal Index proposed by Word [36], and the Feeding Structure Index (FSI) [37] that are based on the different feeding strategies of the benthic organisms. Another example is the Nematodes/Copepods Index [38], which accounts for the different behaviour of two taxonomic groups under environmental stress situations. However, several authors rejected them due to their dependency on parameters like depth, sediments particle size, and due to their unpredictable pattern of variation regarding pollution type [39]. Latter, other proposals appeared, such as the Meiobenthic Pollution Index [40], the Mollusc Mortality Index [41], the Polychaetes/Amphipods Ratio [42], or the index of r/K strategies proposed by De Boer et al. [43], which considers all benthic taxa (although emphasising the difficulty of scoring exactly each species through the biological trait analysis).

On the other hand, Feldman's R/P Index (in [19]), based on marine vegetation, is highly used in the Mediterranean Sea. It was established as a biogeographical index and it is based on the fact that the number of species of Rodophyceae decreases from the Tropics to the Poles. Its application as indicator holds on the higher or lower sensitivity to disturbances of Phaeophyceae and Rhodophyceae.

1.3.3. Indices based on the diversity value

Diversity is another widely used concept focusing on the fact that the relationship between diversity and disturbances can be seen as a decrease in the first one as the stress increases. Nevertheless, some difficulties arise as higher diversity values (in terms of number of species and equitability) are reached under a moderate stress.

Magurran [44] divides diversity measurements into three main categories: (a) indices that measure the increase in species number, such as the Margalef's one, (b) the species abundance models, as the *K*-dominance curves [45] or the log normal model [46], and (c) indices based on the proportional abundance of species, that pretend to solve the increase in number of species and uniformity in a simple expression. This last category of indices can also be divided into those based on statistics, information theory, and dominance indices. Indices derived from the information theory, as the Shannon–Wiener, are based on a logical assumption: the diversity, or information in a natural system, can be measured in a similar way as information contained in a code or message. On the other hand, dominance indices such as the Simpson or Berger–Parker ones are referred as measurements that ponder the abundance of the most common species, instead of the increase in species number.

Meanwhile, average taxonomic diversity and distinctness measures were proposed by Warwick and Clarke [47] to evaluate biodiversity in marine environments, as they take into account taxonomical, numerical, ecological, genetic, and phylogenetical aspects of diversity. These measures address some of the problems identified with species richness, as its dependency on sample size/effort, lack of phylogenetical diversity expression, and non-monotonic behaviour to environmental degradation [48].

1.3.4. Indicators based on species biomass and abundance

Other approaches account for the variation of organism's biomass as a measure of environmental disturbances. Along these lines, there are methods such as species–abundance–biomass–curves (SAB) [49], consisting of a comparison between the curves resulting from ranking the species as a function of their representativeness in terms of abundance and biomass. The abundance–biomass curves (ABC) method [50] also involves the comparison between the cumulative curves of species biomass and abundance, from which Warwick and Clarke [51] derived the *W*-Statistic Index. Although, both methods are helpful to compare data collected from different areas, and to track changes and trends in biodiversity, it is more advisable the use of the ABC method. In fact, through the *W*-Statistic it is possible to relate the index numerical value with environmental factors.

1.3.5. Indicators integrating environmental information

From a more holistic point of view, some authors proposed indices able to potentially integrate the whole environmental information. Satmasjadis [52] proposed a first approach to implement these metrics in coastal areas. The author related the sediment particles size to the diversity of benthic organisms. Moreover, Wollenweider et al. [53] developed a Trophic Index (TRIX) integrating chlorophyll-*a*, oxygen saturation, and total nitrogen and phosphorus concentrations, in order to characterise the trophic state of coastal waters.

In a progressively more complex way, other indices such as the Index of Biotic Integrity (IBI) for coastal systems [54], the Benthic Index of Environmental Condition [55,56] or the Chesapeake Bay B-BI Index [57] (subsequently modified by Van Dolah et al. [58]), included physicochemical factors, diversity measures, specific richness, taxonomical composition, and the trophic structure of the system.

Similarly, a set of fish communities' specific indices was developed to measure the ecological status of estuarine areas. The Estuarine Biological Health Index (BHI) [59] combines two separate measures (health and importance) into a single index. The estuarine Fish Health Index (FHI) [60] is based on both qualitative and quantitative comparisons with a reference fish community. The Estuarine Biotic Integrity Index (EBI) [61] reflects the relationship between anthropogenic alterations in the ecosystem and the higher trophic levels status, and the Estuarine Fish Importance Rating (FIR) is based on a scoring system of seven criteria that reflect the potential importance of estuaries to the associated fish species. This index is able to provide a ranking, based on the importance of each estuary and helps to identify the systems with major importance for fish conservation.

1.3.6. Ecological indicators thermodynamically oriented or based on network analysis

In the last two decades, several functions have been proposed as holistic ecological indicators, intending (a) to express emergent properties of ecosystems arising from self-organisation processes in the run of their development, and (b) to act as orientors (goal functions) in models development, as already referred above. Such proposals resulted from a wider application of theoretical concepts, following the assumption that it is possible to develop a theoretical framework able to explain ecological observations, rules, and correlations on basis of an accepted pattern of ecosystem theories [62]. That is the case of Ascendency [63] and Emergy [64], both originated in the field of network analysis, which appear to constitute suitable system-oriented characteristics for natural tendencies of ecosystems development [65]. Also Exergy [66], a concept derived from thermodynamics, and can be seen as energy with a build in measure of quality, has been tested in several studies (e.g. [67–70]).

1.4. Key for selecting ecological indicators

In the process of selecting an ecological indicator, type of pollution, type of community, data requirements and data availability must be accounted for. Moreover, the complementary use of different indices or methods based on different ecological principles is highly recommended in determining the environmental quality status of an ecosystem [71].

Our proposal is to use a key table (Table 1) to select the most adequate ecological index, being focused on the application of those indices that are most commonly used in marine studies. In the case of coastal and transitional waters ecosystems, there is a clear preference for focusing on benthic invertebrate fauna. In fact, benthic communities integrate environmental conditions and changes occurred through time in a very effective way, allowing therefore to account for the types of disturbances that occur most often in coastal areas (organic enrichment, physical disturbance or toxic pollution), as well as for the level of taxonomic identification of the different organisms.

2. Material and methods

To illustrate the application of this key, data from four study areas (one of them located in the Western coast of Portugal and three areas located at the South-Eastern coast of Spain) were used.

Table 1
Key for selecting ecological indicators

Type of disturbance	Type of data	Ecological indicators
Organic enrichment	Biological data available refers to benthic meiofauna community	(6) (7) (12) (13) (14) (15) (16) (17) (18)
Physical disturbance	Biological data available refers to benthic macrofauna community and the type of data available includes information on physical–chemical parameters, numeric density and organisms' biomass. The organisms are identified up to species level	(1) (2) (3) (4) (5) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22) (23) (24)
Toxic pollution (effects are to be evaluated at the level of communities structure)		
	Biological data available refers to benthic macrofauna community and the type of data available includes information on physical–chemical parameters, numeric density and organisms' biomass. The organisms are identified up to genus or family level	(5) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22) (24)
	Biological data available refers to benthic macrofauna community and the type of data accounts only for organisms' numerical density and biomass. The organisms are identified up to species level	(1) (2) (3) (4) (5) (8) (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22)
	Biological data available refers to benthic macrofauna community and the type of data accounts only for organisms' numerical density and biomass. The organisms are identified up to genus or family level	(5) (8) (9) (10) (11) (12)) (13) (14) (15) (16) (17) (18) (19) (20) (21) (22)
Toxic pollution (effects are to be evaluated at the contaminant's toxic potential)	Contamination is caused by PAHs	(25) (26) (27)
	Contamination is caused by heavy metals	(28) (29) (30)
	Contamination is caused by xenobiotics	(29) (30)
	Contamination is caused by organotin	(31) (32) (33)

(1) AMBI, (2) BENTIX, (3) Indicator Species Index, (4) Pollution Index (Bellan), (5) Pollution Index (Bellan-Santini), (6) Nematods/Copepods Ratio, (7) Meiobenthic Pollution Index, (8) Infaunal Trophic Index, (9) Amphipods/Polychaetes Ratio, (10) Feeding Structure Index, (11) Mollusc Mortality Index, (12) Shannon–Wiener Index, (13) Pielou Index, (14) Margalef Index, (15) Berger–Parker Index, (16) Simpson Index, (17) Taxonomic Diversity, (18) Taxonomic Distinctness Measures, (19) *W*–Statistics, (20) Exergy, (21) Specific Exergy, (22) Pollution Coefficient, (23) B–BI, (24) Benthic Index of Environmental Condition, (25) Bulky DNA Adduct Method, (26) PHA Bile Metabolites Method, (27) Early Toxicopathic Lesions Method, (28) Metallothionein Induction Method, (29) Lysosomal Stability Method, (30) Lysosomal Neutral Red Retention Method, (31) Shell Thickening Method, (32) Imposex Method and (33) Intersex Method.

Each one of the study areas represents a different disturbance scenario. At the same time, the available data are also of different nature. In fact, whereas quantitative data exist for some areas, including comprehensive lists of species and their corresponding abundances and biomass, in other cases only data on the most representative taxonomic groups were available. Having at our disposal such a heterogeneous set of raw data represented an excellent opportunity to apply the proposed key.

The description of the areas as follows:

(a) The Mondego estuary is located in the western coast of Portugal (Fig. 1). This estuary is under severe environmental stress, and changes resulting from an ongoing eutrophication process have been monitored during the last decade. The selected data set was provided by a study on the subtidal soft bottom communities, during spring in both 1998 and 2000, at 14 sampling stations covering the whole system. The study characterised the ecosystem with regard to species composition and abundance, taking into account its spatial distribution in relation to the water physicochemical factors.

(b) Three areas located at the south-east coast of Spain (Fig. 2): the Mar Menor coastal lagoon (Fig. 2A), a 135 km^2 Mediterranean coastal lagoon, the Escombreras basin (Fig. 2B), and the Cape Tiñoso (Fig. 2C). In the Mar Menor case data from Pérez-Ruzafa [72] were used, once they are a complete characterisation of the lagoon benthic populations. In Escombreras basin, data collected at 11 sampling stations, in July 1994, were selected, describing the subtidal soft-bottom communities of the whole system with regard to species composition and abundance. This area presented a high pollution level, originated in waste and industrial dumping that have led to changes in water and sediment physical and chemical characteristics [73].

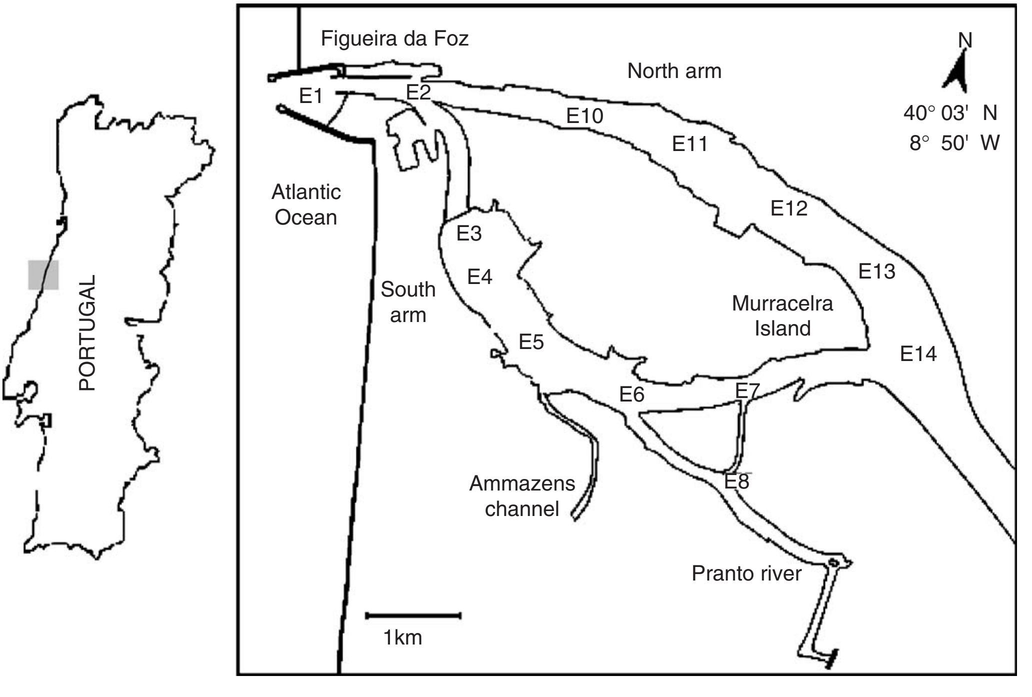

Fig. 1. The Mondego estuary. Location of the subtidal sampling stations.

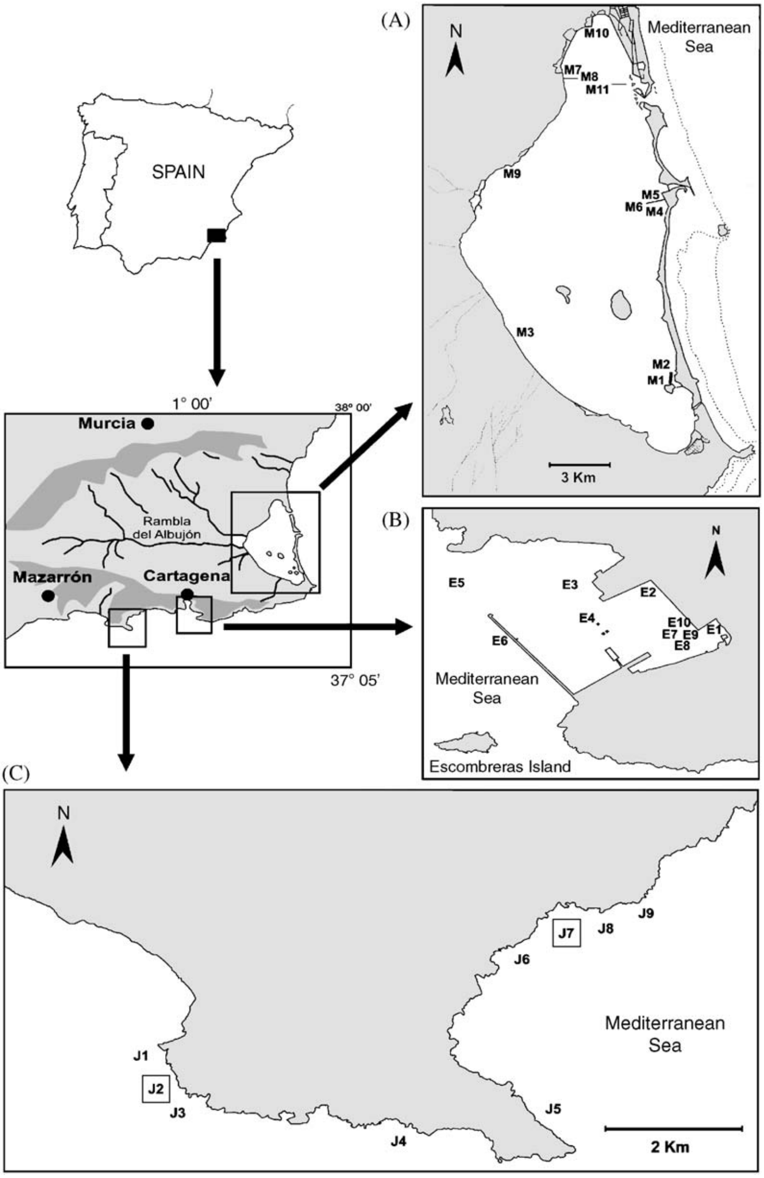

Fig. 2. The Mar Menor coastal lagoon (A), Escombreras basin (B), Cape Tiñoso (C). Location of the sampling stations.

The data from Cape Tiñoso were obtained as a result of a monitoring plan to evaluate the environmental impact caused by fish farming activities devoted to red tuna fattening [74].

The first two things to bear in mind before applying the proposed key are: (a) what is the type of disturbance we want to measure and (b) what type of data do we have? Table 2

Table 2
Summary of the main disturbance factors and the type of data available regarding each one of the four case studies

Study area	Disturbance factor	Type of data
Mondego estuary	Organic enrichment	Abundance of benthic organisms Biomass of benthic organism data available only in 1998 and 2000 Physical–chemical parameters: temperature, salinity, Chl-*a*, nutrients, granulometry, % organic matter
Mira estuary	Unknown	Abundance of benthic organisms
Mar Menor	Organic enrichment	Abundance of benthic organisms Biomass of benthic organism Physical–chemical parameters: temperature, salinity, granulometry, % organic matter, heavy metals concentration in sediments
Escombreras basin	Toxic pollution	Abundance of polychaeta species Biomass of polychaeta species Physical–chemical parameters: temperature, salinity, nutrients, granulometry, % organic matter, heavy metals concentration in sediments
Cape Tiñoso	Organic enrichment	Abundance of polychaeta species Biomass of polychaeta species Physical–chemical parameters: nutrients, Chl-*a*, granulometry, % organic matter, heavy metals concentration in sediments

provides a summary of the main disturbance factors and the type of data available regarding each one of the four case studies.

The next step is to choose the most appropriate indicators according to the nature of the available data, meaning on organisms abundance only or on abundance and biomass, if the species are identified up to species level, etc. Additionally, it must taken into account that among indicators based on the same principles, the ones which best include the characteristics that define a good ecological indicator should be chosen.

For instance, among the indices based on indicator species, we have the pollution indices of Bellan and Bellan–Santini, the BENTIX, the ISI and the AMBI. Out of this panoply, the most appropriate one, as being based on the classification of 3000 species and having been successfully tested in a higher number of geographical locations, is AMBI. On the top of that, the fact that the authors provide free available computer software for its application certainly makes it the most suitable. In fact, BENTIX is too much specific for Mediterranean coastal waters, mainly for areas near Greece, and the ISI is specific for Norwegian and Sweden coastal waters.

If we account for most of the integrating indices, apart from being specific for given estuarine systems, most of the time they have been developed for a concrete sampling area. Among the ones referred in the proposed key, the Weisberg's B-IBI Index is the most popular, and at the same time the one that can be more easily exported to other study areas, constituting therefore the most recommendable to be used.

3. Results of the application

3.1. Mondego estuary

Of all the indicators utilised, the only ones able to discriminate between different areas in the Mondego estuary were the Margalef Index, the Total Taxonomic Distinctness (TTD), the FSI and the AMBI indices (Table 3). More precisely, the Margalef Index and the TTD, highly correlated ($r = +0.91$; $p < 0.001$) were only able to differentiate stations in the North arm from those in the South one. In his turn, AMBI distinguished three groups: (a) stations in the South arm (with higher percentage of organic matter in the sediment), which presented the highest values for the index (indicating greater disturbance), (b) stations in the North arm affected by dredging activities, and (c) less disturbed stations located in the North arm. These last two groups were also differentiated by the FSI.

The discrimination of different areas by AMBI is fundamentally due to the dominance of ecological groups III, IV and V in the South arm stations presenting higher content in organic matter in the sediment (mainly stations E8 and E9). On the other hand, in the North arm stations, species of groups II and III were prevalent, although species from group IV have started to appear since 1998. Still, regarding the North arm, groups I, II and III dominate in the stations less affected by environmental stress.

Despite the high correlation found between AMBI and the B-BI Index ($r = -0.61$; $p < 0.01$), the last is not effective in discriminating different areas. AMBI values also appeared negatively correlated with specific exergy ($r = -0.67$; $p < 0.05$). This suggests that specific exergy, in this case, was expressing the dominance of taxonomic groups usually absent in environmentally stressed situations.

Table 3
Discrimination between different areas in the Mondego estuary based on the values estimated for ecological indicators (Kruskal–Wallis test)

	Average			
	AMBI	FSI	Margalef	TTD
North arm-DA	2.53	0.06	0.87	726.38
North arm-N	2.44	0.36	0.94	432.65
South arm-S	2.76	0.21	0.64	788.50
South arm-OM	3.08	0.19	0.32	610.12
Groups	1-DA, N, S 2-S-OM 3-OM	1-DA, S, OM 2-N, S, OM	1-DA, N 2-S, OM	1-DA,S, OM 2-DA,N, OM

N: Non-dredged areas in the North arm; DA: Dredged areas in the North arm; S: South arm areas with organic matter levels $< 5\%$; OM: South arm areas with organic matter levels $> 5\%$; FSI: Feeding Structure Index; TTD: Total Taxonomic Distinctness.

Table 4
Pearson correlations between the indicators' values and physicochemical parameters in the Mondego estuary

	NO_2^-	NO_3^-	PO_4	NH_4^+	Chl-*a*
Berger–Parker	0.27	0.60	0.45	0.95*	−0.20
Average taxonomic distinctness	0.77*	0.43	0.63*	−0.20	−0.77*
Exergy	−0.68*	−0.67*	−0.80*	−0.36	0.48

*$p<0.05$.

None of the indicators was able to stand out significant differences between different years. Nevertheless, all of them indicated an environmental status improvement in 2000, which coincided with the implementation of mitigation practices since 1998.

Regarding the connexion between the physicochemical environmental factors and the variation of ecological indicators' values, the Berger–Parker Index, the Exergy Index, and the average taxonomic distinctness were the only ones indices sensitive to parameters normally associated with eutrophication (Table 4).

3.2. The mar menor coastal lagoon

In the Mar Menor case study, the different environmental parameters values showed that the areas mostly affected by organic enrichment correspond to stations M2 and M6, where organic matter content in sediments reached values higher than 5%. In both stations Polychaetes were the dominant taxonomic group, being *Heteromastus filiformis* the most abundant species. Was, therefore, expected, the occurrence of lower values of Exergy and Specific Exergy, of diversity measures, and *W*-Statistic, as well as higher values regarding AMBI, the Polychaetes/Amphipods Index, FSI, and the Infaunal Trophic Index. This was, in fact, confirmed for all indicators in station M6, but not in Station M2, where only the *W*-Statistic, the Margalef Index, the TTD and AMBI indicated disturbance (Table 5).

Moreover, the Margalef Index and TTD were the only indicators able to detect significant differences between organically enriched and non-enriched areas ($p<0.05$). The AMBI values were similar in all sampling stations, indicating moderate disturbance in M2 and M6 and slight disturbance in the other ones. Nevertheless, AMBI presented a positive response, although not significant ($r=+0.41$; $p>0.05$), in relation to the organic matter content in the sediments.

In his turn, the *W*-Statistic gave rather confusing results, namely, station M2, which presented lower organic matter contents in the sediments than M6, was classified as the most polluted one ($W=-0.3$).

In general terms, a similar variation pattern was observed with regard to diversity measures and the Exergy Index, which showed positive and significant correlations ($p<0.05$). On the other hand, these indicators were also negatively and significantly correlated ($p<0.05$) with the organic matter content in the sediments, as well as with other structuring factors in the system (e.g. salinity or, in the case of Margalef and Shannon–Wiener indices, also with sediments particles size).

Specific exergy showed a clear positive correlation with the presence of certain heavy metals as Pb ($r=+0.89$; $p\leqslant 0.05$) and Zn ($r=+0.71$; $p\leqslant 0.05$), which is not what we

Table 5
Indices values estimated at the different sampling stations (M1–M10) in Mar Menor coastal lagoon

	AMBI	FSI	ITI	P/A	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ^*	Δ^+	TTD	STTD	*W*-Statistic	Exergy	Sp-Ex
M1A	2.16	0.79	58.26	0.02	2.24	0.44	3.72	0.57	0.38	45.03	72.42	87.25	2966.67	403.52	0.44	2885 503 836	149346
M1B	1.50	0.41	48.02	0.39	3.63	0.72	4.91	0.29	0.13	77.22	89.12	82.51	2722.92	532.79	0.17	183671 203	155184
M1C	1.50	0.47	45.59	0.03	2.19	0.43	3.87	0.61	0.40	43.71	72.74	88.15	2996.97	386.82	0.02	546460 384	76725
M1D	1.63	0.38	43.37	0.04	2.43	0.47	4.32	0.57	0.36	49.77	77.22	88.46	3273.15	370.33	0.26	192624 681	70963
M2D	0.10	0.20	33.34		2.75	0.77	2.13	0.28	0.18	56.45	68.89	85.10	1021.21	514.55	−0.3	15762 446	603402
M3D	3.57	0.09	67.46	0.89	2.06	0.49	3.20	0.68	0.47	42.48	79.71	77.67	1398.04	418.17	0.25	211020	1592
M4D	0.39	0.50	86.50	0.04	2.14	0.55	2.02	0.39	0.28	63.16	87.15	88.25	1323.81	311.77		14126 661 509	69967
M6A	0	0	66.67				0	1.00	1.00	0	0	0	0	0	0.27	285182	14990
M5B	0.41	0.58	38.91	0.12	2.46	0.49	3.87	0.45	0.27	46.34	63.63	85.25	2727.96	430.36	4.58	899957 796	109861
M5C	0.44	0.67	37.81	0.14	2.55	0.56	3.26	0.42	0.25	46.10	61.49	82.19	1972.46	575.37	4.58	76867 912	102457
M6C	3.72	0.67	66.67	1.88	1.44	0.46	1.78	0.74	0.56	38.33	87.56	87.96	791.67	287.21	0.08	94659	92702
M5D	0.50	0.75	38.32	0.18	1.90	0.40	3.41	0.68	0.49	49.01	95.41	86.41	2246.67	407.63	−0.15	145227 127	94642
M6D	3.78	0	58.61	1.05	1.18	0.42	1.24	0.80	0.64	25.20	70.92	76.98	538.89	621.06	0.13	1555 244	109065
M7C	1.05	1.00	73.87	0	2.00	0.45	2.50	0.62	0.42	41.98	72.67	85.79	1801.67	421.20	−0.01	3249 672 701	94686
M8C	0.49	0.57	46.24	0.45	3.54	0.76	4.19	0.21	0.12	74.96	85.12	87.28	2181.94	407.59	0.11	70381 987	67160
M9	1.36	0.30	45.46		2.71	0.68	3.20	0.47	0.26	51.78	69.85	79.03	1264.44	340.26	−0.11	2523 455	14250
M11	1.69	0.60	72.0	1.26	3.75	0.75	5.12	0.26	0.12	73.76	83.93	79.44	2541.94	399.23	0.24	28713 101	78518
M10C	0.81	0	72.61	0	2.75	0.83	1.76	0.34	0.19	47.24	58.27	59.26	592.59	105.62	0.34	301455 400	70064

FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods ratio; Δ: Taxonomic diversity; Δ^*: Taxonomic distinctness; Δ^+: Average taxonomic distinctness (presence/absence of species); TTD: Total taxonomic distinctness; STTD: Variation in taxonomic distinctness; Sp-Ex: Specific exergy.

should expect. For instance, station M2D, which presented the highest concentration of Pb and Zn, also exhibited the higher value of specific exergy.

Regarding the Exergy Index values, the influence of biomass variations, which are related to numerical changes in the dominant populations under environmental stress, appear to be much more important than variations in the quality of the system's biomass (β factors).

3.3. Escombreras basin

In Escombreras basin, the concentration of organic matter in the sediment was taken into account, in order to test the different ecological indicators discriminatory power. Firstly, according to these criteria, stations E2, E7 and E8 were considered as organically enriched. In none of the cases the indicators were capable to discriminate such groups. In general, results obtained with the different indicators were even contradictory. As a matter of fact, while diversity measures taking into account species abundance suggest a higher disturbance in station E8, diversity measures based on species richness indicate station E10 as the most polluted one (Table 6).

Other indicators, for instance the *W*-Statistic behaviour was similar to diversity measures based on species abundance performance. On the other hand, AMBI indicated station E1 as the most polluted one, due to the dominance of *Polydora ciliata*, a Polychaete belonging to ecological group IV.

Finally, the performance of indicators based on ecological strategies was very divergent not only when compared to each other, but also in comparison to indicators from other groups. In general, none of the indicators present any significant correlation to physicochemical parameters.

Table 6
Values of ecological indicators estimated at the different sampling stations (E1–E10) at Escombreras basin

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
AMBI	3.47	2.33	1.59	3.04	1.00	—	2.45	0.65	2.14	1.29
FSI	0.33	0.60	0.50	0.50	0.67	—	0.38	0.33	0.33	0.67
ITI	70.00	52.23	49.02	57.15	83.34	—	50.38	89.72	81.77	90.48
Shannon–Wiener	2.21	3.02	1.33	2.28	1.79	—	3.45	1.24	1.41	1.38
Pielou	0.64	0.91	0.84	0.76	0.90	—	0.88	0.39	0.50	0.87
Margalef	2.17	2.65	0.71	1.87	1.67	—	3.68	1.76	1.24	1.03
Berger–Parker	0.52	0.22	0.58	0.42	0.50	—	0.20	0.78	0.50	0.57
Simpson	0.33	0.11	0.41	0.26	0.20	—	0.09	0.63	0.44	0.33
Δ	38.63	56.63	39.22	46.57	53.33	—	58.16	24.51	37.44	38.10
Δ^*	57.53	63.98	66.67	62.56	66.67	—	64.05	66.09	66.61	57.14
Δ^+	62.42	62.59	66.67	64.29	66.67		63.49	56.94	61.11	55.56
TTD	686.67	625.93	0	514.29	266.67	—	952.38	512.5	427.78	166.67
STTD	113.31	100.69	0	53.85	0	—	79.87	175.54	88.18	246.91
W-Statistic	−0.12	0.49	0.48	−0.02	0.50	—	0.56	−0.18	−0.08	0.12

FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; Δ: Taxonomic Diversity; Δ^*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness (presence/absence of species); TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

Table 7
Pearson correlations between the values of the different ecological indicators estimated based on data proceeding from sampling stations at Cape Tiñoso

	Margalef	Δ^+	Δ^*	Δ	Pielou	STTD	TTD	Shannon	Simpson	Berger
Δ^+	0.15									
Δ^*	0.04	0.75^{**}								
Δ	0.74^{*}	0.42	0.16							
Pielou	0.46	0.24	−0.07	0.84^{*}						
STTD	0.12	-0.56^{*}	−0.56	−0.02	-0.03					
TTD	0.95^{**}	0.19	0.15	0.61^{*}	0.26	0.08				
Shannon	0.96^{**}	0.13	−0.02	0.82^{**}	0.55^{*}	0.18	0.90^{**}			
Simpson	-0.75^{*}	−0.11	0.14	-0.94^{**}	-0.86^{**}	−0.21	-0.58^{*}	-0.85^{**}		
Berger	-0.71^{*}	−0.32	0.24	-0.91^{**}	-0.84^{**}	0.23	-0.59^{*}	-0.83^{**}	0.98^{**}	
W-Statistic	-0.55^{*}	0.23	0.34	0.67^{*}	0.76	0.34	0.45^{*}	0.76	0.56^{*}	0.57^{*}

Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness $*p \leqslant 0.05$; $**p \leqslant 0.01$.

3.4. *Cape Tiñoso*

In the Cape Tiñoso case study, the populations' response to situations of disturbance vs. non-disturbance was tested. Stations J4 and J5, the farthest ones from the cages' influence, were considered as representing the reference situation. Also, samples carried out in August 1996, prior to the floating cages placement, were considered as representing a pristine situation, in opposition to the other sampling periods.

Only AMBI was able to distinguish between reference and disturbed stations ($p<0.05$). Nevertheless, such differentiation, although statistically significant, showed to be irrelevant in terms of differentiating ecological status. In general, all the stations were identified as "good" sensu Borja et al. [12].

Comparing samples from August 1996 with samples from posterior dates, none of the indices were capable to illustrate the a priori assumed pristine situation, neither to distinguish it from the subsequent periods.

Again, significant correlations were found between diversity measures, taxonomic diversity, and the *W*-statistic, and between the Margalef Index and TTD (Table 7).

On the other hand, regarding the ecological indicators response to environmental parameters, contrarily to what was expected, a positive correlation between several diversity measures, taxonomic diversity, and TTD, and the chlorophyll-*a* concentration in the water column was found. These results suggest that the installation of floating cages assigned to red tuna fattening, at least in the first and a half-year, had a fairly small environmental impact, determining an intermediate disturbance situation, which, in fact, favoured a diversity increase.

4. Discussion and general recommendations

Experience demonstrates that none of the available measures of disturbance should be considered ideal. At least in theory, all ecological indicators accounting for the composition and abundance of biological communities might be useful in detecting the environmental situation of an ecosystem. However, due to the fact that in practice most of them were developed to approach the characteristics of a specific ecosystem, they often lack generality. Others have been criticised or rejected due to their dependence on specific environmental parameters, or because of their unpredictable behaviour depending on the type of environmental stress. The choice of ecological indicators set to use in a particular case is, therefore, a sensible process. The currently proposed table key as the intends to facilitate the work of those responsible for measuring the different disturbances impact, with the intention of applying mitigation or correcting measures needed in a broader management framework.

Nevertheless, some comments and recommendations are necessary when applying some of the referred indices.

4.1. Indices based on species indicators

As a whole, results lead us to think that AMBI is a good tool in detecting pollution. In fact, AMBI's application in the Escombreras basin and Cape Tiñoso case studies has lead to good results. For instance although not as clearly as that, in Cape Tiñoso (only accounting for Polychaetes), AMBI was the only indicator able to differentiate the control stations closer to the floating cages' area.

However, some precautions, already described by Borja et al. [75], have to be taken into account consideration for a correct application. Namely, it is assumed that AMBI's robustness is reduced when only a very small number of taxa (1–3) and/or individuals are found in a sample. Moreover, to avoid ambiguous results, it is preferable to calculate AMBI values for each replicate separately, estimating the average value subsequently. When the percentage of not assigned taxa is elevated ($>20\%$), results must be evaluated with caution.

Some indicators, such as BENTIX, proposed by Simboura and Zenetos [14], have been based on AMBI. These authors modified AMBI by reducing from five to three the number of groups involved in the algorithm, in order to avoid errors in grouping species. However, in view of such modification, BENTIX tends towards extreme values when evaluating a systems' ecological status. This type of response is due to the fact of taking into account only sensitive species (GI) and opportunist species of first and second order.

Other indicators, like the Norwegian ISI, require a previous classification of sensitive values (based on the Hulbert Diversity Index) in the study area. For such a purpose, given a study area, a large number of samples are necessary, which is not easy in most of the cases. In Norway, sensitive values for each species were determined after analysing 1080 samples from Norwegian fjords and coastal waters (1975–2001).

The little robustness of Bentix and difficulties in applying an index like ISI, make AMBI the most useful index based on indicator species in establishing the ecological status, at least for the present. Moreover, it has been tested in a large number of geographical areas and is supplied as user-friendly free-available software, including a continuously updated species list (approximately 3000 taxa presently), which makes it especially convenient.

4.2. Indices based on ecological strategies

Regarding the Polychaetes/Amphipods ratio, many samples did not allow appling it simply due to the absence of amphipods. In such case, the ratio would reflect an extremely polluted scenario, which, for certain, was not the case. Although this indicator has been successfully used to detect the effects of organic and oil pollution on subtidal communities at the Bay of Morlaix (Mediterranean Sea) and at the Ría de Ares and Betanzos (Atlantic Ocean), in the present studies it did not work well. As, for instance, the Nematodes/Copepods ratio, which is used in relation to meiobenthic communities, the Polychaetes/Amphipods ratio is probably influenced by a large spectrum of ecological factors, including some types of pollution. This means that this oversimplifying ratio is inadequate and difficult to relate to environmental quality.

Regarding indicators based on the trophic strategies (FSI and Infaunal Trophic Index), the current results have shown their inefficacy as reliable tools in detecting pollution. However, out of the two, the FSI was more efficacious. In fact, while at least FSI allowed discriminating between dredged and non-dredged areas in the Mondego estuary, the ITI was always inefficient in pointing up disturbance situations.

Also, in the Mar Menor coastal lagoon and the Mondego estuary case studies, contrarily to what could be expected in accordance to Word [36], ITI exhibited the highest values precisely in the less organically enriched areas.

Other reasons support the exclusion of this index from the recommendation. One of the disadvantages of this type of indicators is the need for determining the organism's diet, which can only be achieved through the study of stomach's contents, in laboratory experiments, or through stable isotopes analysis. As a rule, the real diet is difficult to establish, and can vary between different populations from the same taxonomic entity. Examples of such ambiguity took place when applying these indicators to data from our study areas. *Nereis virens*, for instance, which is known as an omnivore species along the European coast, turns herbivore in the North American coasts [76]. Moreover, while Word [36] classifies most of the carnivore species as surface detrital feeders, Codling and Asley [77] consider that they should be surface deposit feeders, as most of them consume particles bigger than 50 μm in size. And finally, the literature reports many examples that lead to doubts about the existence of a clear separation between different feeding strategies (e.g. [78–81]). Some of these questions, and the fact that Word's trophic groups' classification is not only based on where the food is captured, but on the size of the particle ingested, make the index even more difficult to apply in environmental studies.

4.3. *Biodiversity as reflected in diversity measures*

Regarding diversity measures, the Margalef Index was the one showing the best performance, despite its relatively simplicity as compared to other indices, namely the ones accounting for species richness and individuals abundance. Actually, it was effective in detecting organic enrichment situations in the Mar Menor lagoon. As for the Shannon–Wiener, Simpson and Berger–Parker indices appear to be much influenced by the dominance of given species (e.g. *Hydrobia ulvae* in the Mondego estuary or *Bittium* sp. in Mar Menor), whose abundance has no relation with any type of disturbance, rather being favoured by abundant food resources.

Out of all the indicators based on Taxonomic Distinctness, only TTD was able to correctly distinguish between different scenarios, (together with AMBI and the Margalef Index) and was able to discriminate between more and less organically enriched areas in Mar Menor. Nonetheless, Warwick and Clarke [48] consider not recommendable the use of that measure due to, in general, it tends to track species richness rather closely, and it is only useful for tightly controlled designs in which effort is identical for the samples being compared, or sampling is sufficiently exhaustive for the asymptote of the species-area curve to have been reached. Although in theory the Taxonomic Diversity measures cover many of the features (e.g. independency on sample size/effort or monotonic response to environmental degradation) required in order to be a good diversity indicator, in view of our results, with the exception of TTD, all the other measures proposed by Warwick and Clarke [47] did not show any advantage as compared to other diversity indices, fact already reported by Hall and Greenstreet [84]. An exemption was the Cape Tiñoso case study, where was possible to observe a situation of intermediate organic enrichment that favoured a diversity increase. Here, contrarily to what happened with other diversity indices, Average Taxonomic Distinctness did not show a positive correlation with the chlorophyll-*a* concentration in the water column, exhibiting in fact a monotonic answer to stress.

Interestingly, the two indices based on specific richness (Margalef Index and TTD) were the most successful measures in differentiating the diverse disturbance levels. This fact, lead us to think that the increment or decrement in the number of species is one of the best disturbance indicators, and therefore, essential to consider when it comes to differentiating ecological status. The inconvenience of these measures, contrarily to Taxonomic Distinctness, is that they may be more sensitive to underlying variation in natural environmental factors, thus generating confounding effects if one is interested in the influence of anthropogenic perturbations [83]. However, studies like Heino et al. [84] showed that Taxonomic Distinctness also varies along natural gradients, being difficult to determine if a site is degraded or not degraded based only on Taxonomic Distinctness measures.

4.4. Indicators based on species biomass and abundance

Most times, in the present case studies, the *W*-Statistic appeared significantly correlated with the Shannon–Wiener, Pielou, Berger–Parker, and Simpson indices, but it presented a clear comparative advantage: its application does not depend on previously known reference values.

It is well documented the dominance of few species with small-size individuals in polluted environments. Nevertheless, this dominance may occur in non-polluted environments [85–88], which may lead to erroneous ecological status assessment. This was probably the reason why the *W*-Statistic was not very successful in detecting organic pollution in the Mar Menor lagoon or at the Escombreras basin, together with the fact that this metric was wholly developed to assess organic pollution impact, and in these two study areas there are also other kinds of pollution (e.g. heavy metals and diffuse pollution stress) besides the organic enrichment.

4.5. Thermodynamically oriented indicators: exergy-based indices

As a whole, our results suggest that the Exergy Index is able to capture useful information about the state of the community. In fact, more than a simple description of the environmental state of a system, the spatial and temporal variations of the Exergy Index may provide us a much better understanding of the system development in the scope of a broader theoretical framework.

However, at the present stage, through simple snapshots, the Exergy Index and Specific Exergy can hardly provide a clear discrimination between disturbed (i.e. polluted) and non-disturbed situations. For instance, in Mar Menor coastal lagoon case study, despite responding to sediment organic enrichment, both the Exergy Index and Specific Exergy were unable to distinguish between areas affected by organic pollution and unpolluted ones.

Besides that, in the case of Mar Menor, it is interesting to note that Specific Exergy appears positively correlated to heavy-metal contamination (such as Lead and Zinc), while the Exergy Index does not, which is basically due to their different responses to biomass variations in the community. In fact, the influence of such variations on Specific Exergy values is far less important, because the weighting factors (expressing the biomass quality) play a major role in the calculations.

4.6. Integrative indices: B-IBI

B-IBI was only applied on the subtidal communities of the Mondego estuary and did not show to be sensitive enough to distinguish between different a priori well-known zones.

Although one of the B-IBI issues is the balance (%) between species sensitive and tolerant to pollution, which should work well, it also takes into account the percentage of trophic groups and diversity, measured by the Shannon–Wiener Index, which, as mentioned before, did not work in distinguishing different levels of eutrophication in the Mondego estuary. Our results appear to indicate that B-IBI is system specific, and therefore its effectiveness depends on the geographical area where it is applied.

In fact, while at the Chesapeake Bay and New York–New Jersey harbour areas the index works satisfactorily, adaptations had to be done to allow its correct application in other areas. For instance, Van Dolah et al. [59] considered four metrics for using B-IBI in Carolina without taking into account diversity values. Possibly, in the Mondego estuary case study it would have been better to bear in mind other types of issues, such as the percentage of abundance of pollution sensitive and pollution tolerant species, and diversity measured as species richness, which proved to work well in this system, instead of considering the proportional abundance of individuals as well. But in that case we would be applying a different index, like the one proposed by Weisberg [57].

Surely, the major inconvenience of an index like B-IBI is the unavoidable need to readapt it to different geographical areas. The basic steps to develop these types of indices are: (a) defining major habitat types based on classification analysis of species composition and evaluation of the physical characteristics of the resulting site groups, (b) selecting a development data set representative of degraded and non-degraded reference sites in each major habitat type, comparing various benthic attributes between them, and (c) establishing a scoring criteria. Obviously, this implies a previous knowledge on the study areas, and the availability of a large database (which in most cases does not exist), in order to validate the measures. Therefore, such constraints lead us to discourage the generalised application of an index of B-IBI type.

Finally, on top of these considerations, except for those based on the presence/absence of indicators species, Salas [89] considered that most of the indices can be applied with similar results independently from the fact that the organisms are identified up to species, genus, or family levels, at least considering organic pollution. Nevertheless, the response of different species from the same Family can vary drastically in the case of moderate physical disturbance and toxic pollution. In that case, results obtained with different taxonomic accuracies will not be equivalent.

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