Fuensanta Salas, Joana Patrício and João Carlos Marques

Ecological Indicators in Coastal and Estuarine Environmental Quality Assessment

A user friendly guide for practitioners

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Index

Chapter 1

INTRODUCTION

1.1. What are indicators and what is their utility?

Ecological indicators are commonly used to provide 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 a system's level.

Indicators are quantitative representations of the forces that drive an ecosystem, of responses to forcing functions, or of previous, current, or future states of an ecosystem. When they are used effectively, indicators are expected to reveal conditions and trends that help in development planning and decision making (Unluata, 1999).

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 (Jørgensen & Bendoricchio, 2001).

Such application proceeds from the fact that conventional models of aquatic ecosystems are not effective in predicting the occurrence of qualitative changes in ecosystems, e.g. shifts in species composition. That is due to the fact that measurements typically carried out, like biomass and production, are not able to capture such modifications (Nielsen, 1995). Nevertheless, it seems possible to incorporate this type of changes in structurally dynamic models (Nielsen, 1992; 1994; 1995; Jørgensen et al., 2002), which allows improving the existing models, not only in the sense of increasing their predictive capability, but also approaching a better understanding of ecosystems behaviour, and consequently a better environmental management.

What happens in structurally dynamic models is that the simulated ecosystem behaviour and development (Nielsen, 1995; Straškraba, 1983) is guided through an optimisation process by changing the model parameters in accordance to a given ecological orientor (goal function). In other words, this allows introducing in models parameters that vary as a function of changing forcing functions and state variables conditions, optimising the model outputs by a stepwise approach. In this case, orientors are assumed to capture a given macroscopic property of the ecosystem, expressing emergent characteristics arising from self organisation processes.

In general, the application of ecological indicators is not exempt of criticisms, the first of which is that 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 kinds of factors, such as physical, biological, ecological, socio-economic, etc. Therefore, indicators must be utilised following the right criteria and in situations that are consistent with its intended use and scope; otherwise they may drive to confusing data interpretations.

1.2. What are the characteristics of a good indicator?

Consider a given indicator as good or less good is, and will always be, a matter of perspective. For instance, from a relatively holistic viewpoint, O'Connor & Dewling (1986) proposed, long time ago, five criteria to define a suitable index of ecosystem degradation, which we think can still be considered up-to-date. An index should be: 1) relevant, 2) simple and easily understood by lawmen, 3) scientifically justifiable, 4) quantitative, and 5) cost-acceptable.

In the very same year, but from a toxicological perspective, Hellawell (1986) detailed the following characteristics as ideal ones for an indicator species: 1) easy to identify and to sample, 2) with universal distribution, 3) having economic importance as resource, 4) easy to cultivate and maintain in laboratory conditions, and 5) exhibiting bio-accumulative ability and low genetic variability. Such features are obviously strictly related to the concept of bio-accumulator.

From the field ecologist perspective, we may say that the characteristics defining a good ecological indicator are (Salas, 2002): 1) handling easiness, 2) sensibility to small variations of environmental stress, 3) independence of reference states, 4) applicability in extensive geographical areas and in the greatest possible number of communities or ecological environments, and 5) relevance to policy and management needs.

UNESCO (2003) also listed the characteristics that environmental indicators should present: 1) to have an agreed scientifically sound meaning, 2) to be representative of an important environmental aspect for the society, 3) to provide valuable information with a readily understandable meaning, 4) to be meaningful to external audiences, 5) to help in focusing information necessary for answering important questions, and 6) to assist decision-making by being efficient and cost-effective in terms of use.

Dale & Beyeler (2001) brought their contribution considering the following as the most suitable qualities of a good ecological indicator: 1) to be easily measured, 2) to be sensitive to stress on the system, 3) to respond to stress in a predictably manner, 4) to predict changes that can be adverted by management actions, 5) to be anticipatory, 6) to be integrative, 7) to have a known response to natural disturbances, anthropogenic stresses and changes over time, and 8) to have low variability in response.

Despite the recognisable convergence of ideas between different authors, it is nevertheless clear that what a good indicator should be does not gather unanimity of opinions. Moreover, it is obviously 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 of stress, or applicable to a particular type of community and/or scale of observation, and rarely its validity has in fact been utterly proved.

3.3. Book structure

This work essentially addresses three tasks spread across five chapters: a) to review the potential indices available to quantify the status of aquatic ecosystems, namely under the European Water Framework Directive scope (EC, 2000), b) to build a decision tree to facilitate which indices to choose in any particular case involving benthic fauna, and c) to evaluate the performance of the various indices in portraying visible qualitative differences among a suite of marine and estuarine ecosystems.

Following the introduction, chapter 2 examines ecological indicators and their characteristics. It includes brief references to terrestrial and freshwater ecological indicators and a comprehensive review of those applied in assessing coastal and marine environments, considering six groups: a) indices based on indicator species, b) indices based on ecological strategies, c) indices based on diversity, d) indicators based on species biomass and abundance, e) indicators integrating all environmental information, and f) indicators thermodynamically oriented or based on network analysis. Chapter 3 provides a decision tree for selecting ecological indicators as a function of benthic fauna data type and availability. Chapter 4 shows how this decision tree was applied in practice in different case studies. Finally, chapter 5 discusses how to combine indicators when characterising systems' ecological status.

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CHAPTER 2

REVIEW OF ECOLOGICAL INDICATORS AND THEIR CHARACTERISTICS

With regard to terrestrial environments, as well as the aquatic ones (fresh or marine) there are numerous ecological indicators designed to measure ecosystems' health. To carry out a comprehensive review of all of them is certainly out of our range, but the development of a guide concerning the right use of such indices, taking as examples some of those environments, will constitute a good tool for further works regarding its application in environmental management. In the present work, although the most commonly applied terrestrial and freshwater ecological indicators are concisely visited, we have chosen to concentrate on those applied in the environmental quality assessment of estuaries and marine ecosystems.

Most of the ecological indicators used and/or tested in evaluating the health status of marine and transitional waters ecosystems can be found in the literature, resulting all of them from just a few distinct theoretical approaches. A first group of indicators focus on the presence/absence of given indicator species, while others take into account the different ecological strategies adopted by organisms, diversity, or the energy variation in the ecosystem resulting from changes in the individuals biomass. A second group of indicators is thermodynamically oriented or based on network analysis, looking for capturing the information on the ecosystem from a more holistic perspective. Finally, a third group attempts to include all the information of the environment in one single value, constituting the so called integrity indices.

Indicators based on diversity, as well as those thermodynamically oriented, can be used in all types of systems. On the contrary, indices based on indicator species and ecological strategies, as well as integrity indices, are more often specifically designed as a function of the environment to be evaluated, despite the fact of sharing the same conceptual bases.

2.1. Brief reference to terrestrial ecological indicators

The seek for biological indicators of disturbance in terrestrial environments has been undertaken in different directions (Blair, 1996; Mason, 1996; McGeoch, 1998). For instance, terrestrial invertebrates are good indicators because they are ubiquitous, diverse, easy to sample, and ecologically important (Andersen, 1997). They play diverse roles in natural environments as decomposers, predators, parasites, herbivores and pollinators, and respond to various perturbations (Price, 1998). Besides, certain taxa such as beetles, butterflies, spiders and ants respond to effects of human or natural disturbance.

Therefore, some groups such as Formicidae (ants) and Carabidae (beetles) have been well studied as indicators of disturbance. Carabid beetles, for instance, respond to agricultural practices, fire, and clearcutting (Refseth, 1980; Holliday, 1991; Niemela *et al.*, 1993). Within this group, different trophic groups show different sensitivity to agricultural management. For instance, it has been observed that carnivore and phytophage taxa richness tends to decrease rapidly with disturbances leading to landscape simplification, while polyphagous taxa migh even increase because of their opportunistic feeding habitats and higher tolerance to disturbance (Purtauf *et al.*, 2005).

Ants have been used in Australia and USA in monitoring programmes associated with mining, fire, grazing and logging, and after several authors (*e.g.* Majer *et al.*, 1984; Neumann, 1992; Andersen, 1997; Nash *et al.*, 1998) taxa richness is higher in some types of disturbed sites. Spiders, in his turn, are affected by vegetation architecture and prey availability (McIver *et al.*, 1992). However, some spiders such as wolf spiders are better adapted to disturbance because the fact of carrying their egg sacs allows them the colonization of disturbed areas (Uetz, 1976). Lepidoptera include taxa with diverse trophic roles (Hammond & Miller, 1998) and on the basis of several studies (*e.g.* Holl, 1996; Spitzer *et al.*, 1997) it is likely the occurrence of fewer taxa at disturbed sites.

Kimberling *et al.*, (2001) designed a biological integrity index based on terrestrial invertebrates in the shrub-steppe of eastern Washington (USA). This index accounts for the following eight metrics: total number of families, number of Diptera families, relative abundance of detritivores, and taxa richness of Acaria, predators, detritivores, ground-dwellers and polyphagous carabid beetles.

With regard to terrestrial vertebrates, birds have been found to be useful biological indicators because they are ecologically versatile, respond to secondary changes resulting from primary causes, and can be monitored relatively inexpensively (Koskimies, 1989). Also, because of their high mobility, birds react rapidly to changes in their habitat (Morrison, 1986; Fuller *et al.*, 1995, Louette *et al.*, 1995). According to Browder *et al.*, (2002) bird taxa are appropriate indicators for monitoring changes for several reasons: a) individual bird species are associated with particular habitats, b) birds occur across a broad gradient of anthropogenic disturbance, from pristine wilderness to metropolitan areas, c) most bird species live only a few years, so changes in species composition and abundance will manifest relatively quickly after a disturbance, d) groups of bird species can be used to develop associations with habitats that are predictive of the relative level of anthropogenic disturbance, and e) birds are important to a large segment of the public (Szaro, 1986; Canterbury *et al.*, 2000), so the public may better relate to concerns about changes in bird communities than those of other taxa, such as plants or invertebrates.

Browder *et al.* (2002) developed a measure of grassland integrity using presence and abundance of disturbance-intolerant and disturbance-tolerant bird species. This index provides a method of monitoring grassland integrity based on the tolerance of grassland birds to anthropogenic disturbance, particularly cultivation. On the other hand, Reynaud & Thiolouse (2000) used co inertia analysis to identify birds as biological markers along an urban-rural gradient.

Concerning plant communities, vegetation cover is generally used to measure the biological diversity and to detect antropogenic disturbances such as the change from high diversity prairies and late sucessional forest dominated by perennial native species to relatively homogeneous agricultural fields dominated by annual crops and weed species (Delong & Brusven, 1998). Understorey herbs have been used as effective indicators of deciduous forest regeneration in southern Canada (McLachlan & Bazely, 2001), long--term continuity of boreal forest in Sweden (Ohlson *et al.*, 1997), military traffic in longleaf pine forest in Georgia (Dale *et al.*, 2002) and riparian forest disturbance in southern USA (Bratton *et al.*, 1994).

Diversity measures, such as total species richness are often used as indicators of forest changes but require a full characterisation of the forest (Moffatt & McLachlan, 2004). Although the use of individual plants species as indicators may eliminate the need for a full description of forests, they may only yield-site-specific information and reveal little about mechanisms underlying forest change. The use of guilds, groups of species that are functionally related and have similar resource requirements, represents an intermediate solution for describing the impacts of disturbance (Hobbs, 1997). Thus, life history and life form have been used to monitor forest disturbance

(McIntyre *et al.*, 1995; Dale *et al.*, 2002), origin and habitat preference have been related to forest species loss and compositional change associated with urban land use (Drayton & Primack, 1996). Flowering phenology and seed dispersal have been related to species decline associated with human use (McLachlan & Bazely, 2001).

Moffatt & McLachlan (2004) showed that herbaceous species, both individually and grouped according to functional types or guilds, are effective indicators of environmental change and disturbance associated with land use. These authors identified two categories of species response to urban land use: urban exploiters, restricted to or dominant in disturbed urban forests, and urban avoiders, excluded from disturbed urban forests. A third set of plant species appeared in association with both urban and suburban sites, in contrast to a fourth group, more frequentin rural and reference sites. In addition, they observed that most of the indicators of disturbance and opportunistic species were exotic, while nearly all vulnerable species were native, as were all species identified as effective indicators of forests in state of integrity.

On the other hand, life history traits also lie beneath understorey responses to land use. For instance, woody species tend to be more resistant to disturbance, perhaps because of their relatively longer life spans and greater structural durability (Robinson *et al.*, 1994). On the other hand, annuals tend to respond positively to disturbance, in part because of their often rapid-rates in terms of biomass and abundant seed production (Bazzaz, 1986).

Seed dispersal also underlay understorey responses to land use. Indicator species of disturbance tend to be endozoochores that produce berries. Other studies of degraded forests have found that myrmecochores (Dzwonko & Loster, 1992), barcoheres (Matlack, 1994), and ephemerals (McLachlan & Bazely, 2001) are vulnerable to habitat fragmentation and physical disturbances.

Wind-dispersed seeds are also likely to exhibit higher mortality in highly fragmented urban environments because their dispersal patterns are largely non-selective (Van der Pijl, 1972). Dispersal-restricted species (those that are gravity, explosion, or ant-dispersed) often travel only centimetres per year and usually are unable to traverse the large gaps that separate urban patches (Dzwonko & Loster, 1992).

DeKeyser *et al.* (2003) developed and Index of Plant Community Integrity (IPCI) to assess quantitatively the quality of seasonal wetlands' communities. They delineated plant data into the same metrics of the rest of the data set (*e.g.* species richness, percentage of introduced and annual plants) and analysed these metrics using principal components and cluster analyses, which allowed defining five quality classes: Very good, Good, Fair, Poor and Very Poor.

In other cases, some measurements as the Fluctuating Asymmetry (FA) in some vegetal species such as *Lythrum salicaria* have been used to detect heavy metals pollution (Mal *et al.*, 2002). Fluctuating Asymmetry measures the random deviation from perfect bilateral or radially symmetrical morphological traits in a group of organisms (Wilsey *et al.*, 1998; Palmer & Strobeck, 1986). In a bilaterally symmetrical trait, the left side is identical to the right, and deviations from perfect bilateral symmetry can come in the form of directional asymmetry, antisymmetry, or FA (Palmer, 1994; Leary & Allendorf, 1989). Of these three kinds of asymmetry, only FA is thought to be caused by developmental noise or imperfect developmental stability (Palmer, 1994; Palmer & Strobeck, 1986) and several studies have shown that is related to abiotic stress, noise, nutrition and pollutants (Möller & Swaddle, 1997). In fact, the FA in three species (*Robinia pseudocaia*, *Sorbus aucuparia*, and *Matricaria perforata*) augmented with increased levels of radiation in Chernobyl (Möller, 1998), and increased FA of leaves caused by metal and chemical pollution in the air has also been reported by Zvereva *et al.* (1997) and Kryazheva *et al.* (1996).

Also, functional indices have been used in terrestrial ecosystems. A food web approach to disturbance and ecosystems' stress was applied by Moore & de Ruiter (1997 a; b) and food webs and productivity, combined with nutrient cycling, have been used to assess stability and disturbance of soil ecosystems and agro-systems in a number of cases (e. g. Moore & de Ruiter, 1991; Moore *et al.*, 1993; de Ruiter *et al.*, 1994; 1995).

2.2. Brief reference to freshwater ecological indicators

With regard to freshwater aquatic environments, the use of biotic indices is the most common approach in assessing the quality of a river or lake. There is no doubt that the most well know index in Europe is Hellawell's (1986) BMWP (Biological Monitoring Working Party), and its subsequent modifications accounting for the taxa found in the geographical areas where it was applied. For instance, in the Iberian Peninsula, the index was modified by Alba & Sánchez (1988) and named Iberian Biological Monitoring Working Party or IBMWP. The index is computed adding the ponctuations attributed to the different taxa found in macroinvertebrates' samples, which are cited in a list developed with this purpose. The punctuation assigned to a given taxa is proportional to its higher or lower sensitivity to organic pollution and to the level of oxygen deficit usually resulting from that type of pollution in most rivers, with the exception of the most torrential ones, where water agitation determines an higher oxigenation.

Other biotic indices used to assess quality in freshwaters systems are the Saprobic Index (Zelinca & Marvan, 1961 in Resh & Jackson, 1993), which is based on the number and abundance of the taxa included in the saprobic list, the ISO Score (ISO, 1984), calculated as the sum of the tolerance scores for the taxonomic families present, the Belgian Biotic Index Method (De Paw & Vanhooren, 1983), based on the total number of systematic units and number of units in different faunal groups, the Biotic Index (Chutter, 1972;

Hilsenhoff, 1987), which determines a community score by weighting the relative abundance of each taxon in terms of its tolerance to pollution, and the Florida Index (Ross & Jones, 1979), that accounts for pollution tolerance of different taxa, although unlike some biotic indices it only considers taxonomic richness, disregarding taxa relative abundances.

With regard to indices based on ecological strategies used in freshwater systems, we may refer: a) the ratio of shredders in relation to the total number of individuals (Plafkin *et al.*, 1989), based on the assumption that shredder organisms and their microbial food base are sensitive to toxicants and to modification of the riparian zone; b) the ratio of scrappers to collector-filterers, which assumes that collector-filterers dominance may reflect organic enrichment; c) the ratio of trophic specialists in relation to generalists (Maine Department of Environmental Protection, 1987), that considers trophic generalists to be more pollution-tolerant, thus becoming numerically dominant in response to environmental stress; and d) the ratio of EPT abundance in relation to Chironomidae abundance, which accounts for the fact that Chironomidae are perceived to be pollution-tolerant as compared to the pollution-sensitive Ephemeroptera, Plecoptera, and Trichopera. Compared with a non-stressed habitat, a stressed one will show an unbalanced composition regarding these groups (Resh & Jackson, 1993).

Among the indices most widely used to measure the biological diversity in rivers, one of the most interesting is the Fractal Dimension of Biocenosis (D), proposed by Margalef (1991), which was originally developed by Docampo & Bikuña (1991) as a biological index to be applied in assessing river communities. Its present formulation expresses the speed in identifying the benthic invertebrate's species or whatever other taxocenosis when the size of the biological sample increases (number of collected individuals or number of analysed individuals), according to the following equation $LogS/LogN$ where S is the richness in species, or alternatively, the taxonomic richness; and *N* is the number of individuals. In non polluted rivers it holds an average value of 0.385 and decreases strongly in rivers impacted by human impacts.

Often, there is the need to determine the phyto-physiological status of fluvial stretches in terms of quality diagnosis accounting for the different behaviours of water masses as a function of their response to the increment of primary producers (microphyates and macrophytes). This assessment is carried out according to which of the two phyto-physiological types considered in river ecosystems they belong. These two types, photosystem I and photosystem II, are established on the basis of photo-pigments' concentration, that is used to diagnose the phyto-physiological status of the river system.

Photosystem I is characterised by a high value of the a/b index on *Cladophora*, which implies a high algae dominance in the fluvial ecosystem, an oversaturation state of chlorophyll *a* (vegetal biomass), and therefore eutrophication or even hipereutrophication. Photosystem II, on the other hand, is characterised by a low value for the a/b index, which implies natural algae metabolism conditions, with a balanced ratio between production and assimilation in the system. Margalef's pigment index (1989), D430/D665, which measures the relation between the concentration of all the pigments (carotenes, xanthophylls, as well as *a*, *b*, *c*, and *d* chlorophylls) and the concentration of chlorophylls alone can be used to distinguish between the two photosystems, presenting lower values when chlorophyll *a* is predominant (System I), and increasing when the other pigments are well represented (System II) or when chlorophyll *a* degrades, increasing the degradation products, among which pheophytine can be found (Margalef, 1983). The index can therefore show abnormally high values in polluted rivers (Margalef, 1983). However, the Chlorophytes Index, IC, (which expresses the cologarithm of the ratio a/b·f) provides a more clear distinction between both types of photosystems than D430/D665 index. Negative values of IC indicate that the stretch is photosystem I characterised, and positive values indicate photosystem II.

Ecological studies have made clear since long time ago that algae, especially benthic ones, as having a limited capability to move, constitute one of the best indicators of the conservation status and biological quality of the aquatic systems (Lowe & Pan, 1996). Nowadays, several European countries (Poland, Germany, France, Austria, Switzerland and United Kingdom) maintain a control net based on the use of Diatomea. Particularly, the Diatomea Biological Index (IBD) has been developed by the French water agencies with the aim of spreading along the country a method first developed in the Siena basin, and in the Rodane-Mediterranean-Corsica and Artois-Picardie water agencies.

Fish communities are also used in assessing the quality of hydrographic basins, as they are submitted to a wider variety of impacts than benthic macroinvertebrates, *i.e.* species extinction as a consequence of mechanical and physicochemical pollution of the waters, population movements and the enfavouring of alochthon fish species to the detriment of autochthon species due to water nutrient enrichment, reduction in the circulating volume of water, and the canalling of fluvial stretches. Particularly frequent is the risk of competitive exclusion of the autochthon species at local or metapopulational level as derived from the presence of alochthon species (Borja *et al.*, 2003a).

Among the most cited indices based on fish communities, it is worthwhile to refer IBI index (Karr, 1981), which is a biological integrity index designed to measure stream pollution. This index bears parameters such as diversity, abundance, trophic level, population structure, migration vs. resident species, and tolerant vs. sensitive species. Moreover, recently, Borja *et al.* (2003a) have used the ECP index (Fish Conservation Index) to measure the rivers quality in the Basque Country (Spain). Such index includes measurements as the ratio autochthon species/potential autochthon species, the quotient sensitive species/tolerant species and the proportion of pathological individuals.

Other biological integrity indices account for the invertebrates populations. An example is the Invertebrate Community Index (EPA, 1987), which is calculated as the sum of 10 individual measures (total number of taxa, total number of Trichopera taxa, total number of Diptera taxa, percent of Ephemeroptera, percent of Trichophera, percent of the tribe Tanytarsini of the Chironomidae, percent of other dipterants and noninsects, percent of tolerant organisms, and number of Ephemeroptera, Plecoptera, and Trichoptera taxa) that are scored individually. The Mean Biometric Score (Shackleford, 1988) is also a combination of community diversity, indicator organisms, and functional groups approaches, and the Biological Condition Score (Plafkin *et al.*, 1989) is calculated through eight metrics which reflect groups tolerance, community structure, and community function.

2.3. Review of the ecological indicators used in assessing coastal and marine environments

Following the promulgation of the European Water Framework Directive (EC, 2000) the need for stable and comparable criteria in environmental quality assessment of aquatic ecosystems, including coastal zones and estuaries, reactivated the use and search of pollution ecological indicators.

In this review we consider the indices most used to assess pollution effects in transitional waters and coastal areas until the end of 2004. The algorithms of the different indices are provided in full detail, and their application in different scenarios, with regard to the necessary requirements as a function of data quality and availability, will be further approached through a binary key (see Chapter 3).

2.3.1. Indices based on indicator species

Among what are usually denominated indicator species, we may distinguish two different cases, whether they are considered as indicators in the most common sense, or as bioaccumulator species (the latter more appropriate in toxicological studies), which may sometimes lead to confusion. In the first case we are referring to those species which appearance and dominance is associated to an environmental deterioration, because they are favoured for such fact, or because they are more tolerant to that type of pollution than other less resistant species. In this sense, the possibility of assigning a certain grade of pollution to an area in terms of the species present has been pointed out by a number of researchers (Blegvad, 1932 and Filice, 1954 in Planas & Mora, 1987; Glemarec & Hily, 1981), mainly with regard to organic pollution studies. In fact, different authors have focused on the presence/absence of such species to formulate biological indices. For instance, the Bellan Index (based on polychaetes), or the Bellan-Santini Index (based on amphipods), attempt to characterise environmental conditions by analysing the dominance of species indicating some type of pollution in relation to the species considered to indicate an optimal environmental situation (Bellan, 1980; Bellan-Santini, 1980).

Nevertheless, many authors claim that is not advisable the use of such indicators because often the species looked upon may occur naturally in relative high densities. In fact, there is no reliable methodology to know at which level one of those indicator species can be well represented in a community that is not really affected by any kind of pollution, which leads to a significant exercise of subjectivity (Warwick, 1993). Despite these criticisms, even recently, the AMBI Index (Borja *et al.*, 2000), based on the Glemarec & Hily (1981) species classification regarding their response to pollution, as well as the BENTIX Index proposed by Simboura & Zenetos (2002), the Norwegian Indicator Species Index (ISI) (Rygg, 2002) or the

the Benthic Quality Index (BQI) (Rosenberg *et al.*, 2004), all applying the very same principles, have gone back to update such pollution detecting tools. Moreover, Roberts *et al.* (1998) also proposed an index based on macrofauna species which accounts for the ratio of each species abundance in control vs. samples proceeding from stressed areas. This proposal is however semi-quantitative as well as site and pollution type specific. In the same way, the Benthic Response Index (Smith *et al.*, 2001) is based upon the type of species present in a sample (related to pollution tolerance), but its applicability is complex as it is calculated using a two-step process in which ordination analysis is employed to quantify a pollution gradient within a calibration data set.

The AMBI Index, for instance, which accounts for the presence of species indicating a given type of pollution, as well as species indicating a non polluted situation, has been considered very useful in terms of implementing the European Water Framework Directive in coastal ecosystems and estuaries. In fact, although this index is very much based on the paradigm of Pearson & Rosenberg (1978), which emphasises the influence of organic matter enrichment on benthic communities, it has been shown useful to assess other anthropogenic impacts, such as habitat physical disturbance, heavy metals inputs, etc. Moreover, it has been successfully applied in Atlantic (North Sea, Bay of Biscay, and South of Spain) and Mediterranean (Spain and Greece) European coasts (Borja *et al.*, 2000, 2003b, 2003c; Casselli *et al.*, 2003; Forni & Occhipinti Ambrogi, 2003; Nicholson & Hui, 2003; Bonne *et al.*, 2003; Muxika *et al.*, 2003; Gorostiaga *et al.*, 2004; Salas *et al.*, 2004).

Marine benthic macrophytes, in their turn, respond directly to the abiotic and biotic aquatic environments, and thus represent sensitive bioindicators regarding their changes (Orfanidis *et al.*, 2003). On the other hand, a series of algae genera are universally considered to appear when pollution situations occur, such as the green algae *Ulva, Enteromorpha, Cladophora* and *Chaetomorpha* and the red algae *Gracilaria, Porphyra* and *Corallina*.

Moreover, species with high structural complexity, like the Phaeophyta belonging to *Fucus* and *Laminaria* genera, are seen worldwide as the most sensitive to any kind of pollution, even if *Fucus* species may cope with moderate pollution (Niell & Pazó, 1978). Finally, marine Spermatophytae are considered indicator species of good water quality.

In the Mediterranean Sea, for instance, the presence of *Cystoseira* and *Sargassum* (Phaeophyta) or *Posidonia oceanica* meadows indicate good water quality. Thus, monitoring the population density and distribution of such species allows detecting and evaluating the impact of whatever activity (Pérez-Ruzafa, 2003). *Posidonia oceanica* is possibly the most used indicator of water quality in the Mediterranean according to their sensitivity to disturbances, its wide distribution along the Mediterranean coast and the good knowledge about the plant and its ecosystem specific response to a particular impact (*e.g.* Ruiz *et al.*, 2001; Pergent-Martini *et al.*, 2005; Romero *et al.*, 2005) Furthermore, this specie is able to inform about present and past level of trace-metals in the environment (Pergent-Martini, 1998).

Pergent-Martini *et al.* (2005) identified the descriptors of *Posidonia oceanica*, constituting the first step to allow the use of this specie to assess the ecological status of Mediterranean coastal zones (Table 1). On this basis, it was developed a index (POMI, *Posidonia oceanica* Multivariate index) based on those physiological, morphological, and structural descriptors combined into a variable using a PCA (see Romero *et al.*, 2005).

In the same sense, a Conservation Index (Moreno *et al.*, 2001), based on the named marine Spermatophyta, is used in Mediterranean coasts. Along the same lines, Orfanidis *et al.* (2001) introduced a new Ecological Evaluation Index (EEI) to assess ecological status of transitional and coastal waters in accordance to the European Water Framework Directive. This index is based on the the marine benthic macrophytes classification in two ecological state groups (ESGs I,II), representing alternative ecological states (pristine and degradated).

Table 1

Recompilation of the main descriptors of *Posidonia oceanica* (Pergent-Martini *et al.*, 2005).

Descriptor	Measured parameters	Information
Upper depth limit	Depth, localisation, densiy, bottom cover, characterisation of the substrate	Human impact, hydrodynamism, sedimentary dynamics
Density	Number of shoot on a surface >1600 cm^2	Dynamic of the meadow, Human impact
Epiphytic coverage	Biomass, diversity	Nutrients concentrations, flora and fauna biodiversity
Bottom cover	% of meadow on a given surface (1 to 25 m^2)	Dynamic of the meadow, Human impact
Leaf biometry	Type, number, size of leaves, leaf surface, Coeffcient A, biomass, epiphytic coverage, presence of necrosis	State of health of the meadow, Human impact, hydrodynamism, hervibory pressure
Lower depth limit	Depth, localisation, type, density, bottom coverage, leaf biometry, granulometry, content in organic matter	Water transparency, human impact, hydrodynamism, dynamic of the meadow (regression of colonisation)
Population associated to the meadow	Fauna, flora, diversity	Biodiversity, interactions meadow-population
Structure of the matte	Intermattes, «cliff of dead matte», erosive structures, recedind, silting up, biodiversity of the endofauna, homogeneity, resistance and compactness, % plagiotropic rhizomes, width of the matte, physico-chemical composition	Dynamic of the meadow, human impact, sedimentary dynamics, study of currents
Biochemical and chemical composition	Elementary composition (C, N, P) phenolic compounds, proteins, carbohydrates, stress enzymes	Dynamic of the meadow, Human impact, hervibory pressure
Datation measurement	Lepidochronology, plastochrone interval, paleo-flowering, primary production	Temporal evolution of the production, sedimentation speed, intensity of the sexual reproduction, dynamic of the meadow, Human impact
Contamination	Metals (Hg, Cu, Cd, Pb, Zn)	Human impact

A. Measures based on indicator species

2.3.1.1. Annelida Pollution Index (Bellan, 1980):

$$API = \sum \frac{Dominance\ of\ pollution\ indicators}{Dominance\ of\ clean\ water\ indicators}$$

Species considered as pollution indicators by Bellan (1980) are *Platynereis dumerilli*, *Theosthema oerstedii*, *Cirratulus cirratus* and *Dodecaceria concharum*. Species considered as clear waters indicators by Bellan (1980) are *Syllis gracillis*, *Typosyllis prolifera*, *Typosyllis* spp and *Amphiglena mediterranea*.

Index values above 1 show that the community is pollution disturbed. As organic pollution increases, the index values become higher allowing, in theory, to establish different pollution grades, although the author does not define them.

This index was, in principle, designed to be applied on rocky superficial substrates. Nevertheless, Ros *et al.* (1990) modified it in order to be applied to soft bottoms, considering other indicator species. In this case, the pollution indicator species are *Capitella capitata*, *Malococerus fuliginosus* and *Prionospio malmgren*, and the clear water indicator species is *Chone duneri*.

2.3.1.2. Pollution Index (Bellan-Santini, 1980):

This index follows the same formulation and interpretation as the Bellan's one, but takes into account the amphipods group.

$$PI = \sum \frac{Dominance\ of\ pollution\ indicators}{Dominance\ of\ clean\ water\ indicators}$$

As pollution indicator species the author considers *Caprella acutrifans* and *Podocerus variegatus,* and as clear water indicator species *Hyale* sp., *Elasmopus pocillamanus* and *Caprella liparotensis.*

2.3.1.3. AMBI **(Borja *et al.*, 2000):**

To apply AMBI, the soft bottom macrofauna is divided into five groups, according to their sensitivity as a function of an increasing stress gradient:

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.

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

The index is estimated following the given algorithm:

$$AMBI = \frac{\{(0 \times \%\,GI) + (1.5 \times \%\,GII) + (3 \times \%\,GIII) + (4.5 \times \%\,GIV) + (6 \times \%\,GV)\}}{100}$$

Table 2

Categories considered as a function of AMBI index values.

Classification	AMBI value
Normal	0 - 1.2
Slightly polluted	1.2 - 3.2
Moderately Polluted	3.2 - 5
Highly polluted	5 - 6
Very highly polluted	6 - 7

To implement this index, more than 3000 taxa have been classified, representing the most important soft bottom communities present in European estuarine and coastal systems. The Marine Biotic Index can be applied using the AMBI© software (Borja *et al.*, 2003b and **www.azti.es**, where the software is freely available).

2.3.1.4. BENTIX (Simboura & Zenetos, 2002):

This index is based upon AMBI but involves a smaller number of ecological groups in the algorithm, which decreases difficulties in the process of grouping the species, and simultaneously simplifies the calculations. The BENTIX algorithm is given by:

$$BENTIX = \frac{\{(6 \times \% GI) + 2 \times (\% GII + \% GIII)\}}{100}$$

Group I: This group includes species sensitive to disturbance in general.

Group II: Species tolerant to disturbance or stress whose populations may respond to organic enrichment or other source of pollution.

Group III: This group includes the first order opportunistic species (pronounced unbalanced situation), pioneer, colonisers or species tolerant to hypoxia.

A list of indicator species in the Mediterranean Sea was compiled, assigning a score ranging from 1 to 3, corresponding to each one of the three ecological groups.

Five categories are considered as a function of the index values (Table 3).

Table 3

Categories considered as a function of BENTIX index values.

Classification	BENTIX value
Normal	4.5 - 6.0
Slightly polluted	3.5 - 4.5
Moderately Polluted	2.5 - 3.5
Heavily polluted	2.0 - 2.5
Azoic	0

2.3.1.5. Macrofauna Monitoring Index (Roberts *et al.*, 1998):

It is an index addressing the biological monitoring of dredged spoil disposal. Each of twelve indicator species is assigned to a score, taking primarily into account the ratio of its abundance in control *vs*. impacted sites' samples. The index value is the average score of those indicator species present in the sample.

Index values of 0 to 2.2, < 2.2 to 6 and > 6 indicate respectively severe impact, patchy impact, and no impact. Although this index is site and impact specific, the process of developing efficient monitoring tools from an initial impact study should be widely applicable (Roberts *et al.*, 1998).

2.3.1.6. Benthic Response Index (Smith *et al.*, 2001):

The Benthic Response Index (BRI) corresponds to the abundance weighted average pollution tolerance of species occurring in a sample, and is similar to the weighted average approach used in gradient analysis (Goff & Cottam, 1967; Gauch, 1982). The algorithm is:

$$I_s = \frac{\sum_{i=1}^{n} p_i \sqrt[3]{a_{si}}}{\sum_{i=1}^{n} \sqrt[3]{a_{si}}}$$

Where I_s is the index value for sample s, n is the number of species for sample s, p_i is the position for species i on the pollution gradient (pollution tolerance score), and a_{si} is the abundance of species i in sample s.

According to the authors, determining the pollution tolerance score (p_i) for the different species involves four steps: (1) assembling a calibration infaunal data set; (2) carrying out an ordination analysis to place each sample in the calibration set on a pollution gradient; (3) computing the average position of each species along the gradient and (4) standardising and scaling the position to achieve comparability across depth zones.

The average position of species ***I*** (p_i) on the pollution gradient defined in the ordination is computed as:

$$Pi = \frac{\sum_{j=1}^{t} g_j}{t}$$

Where ***t*** is the number of samples to be used in the sum, with only the highest ***t*** species abundance values included in the sum. The g_j is the position on the pollution gradient in the ordination space for sample ***j***.

This index has only been applied for assessing benthic infaunal communities on the Mayland Shelf of Southern California employing a 717-sample calibration data set.

2.3.1.7. Indicator Species Index **(Rygg, 2002):**

The index is calculated taking into account the average of sensitivity values corresponding to the species occurring in a sample, based on the approach developed by Rygg (1985). The author identified positive indicator species (pollution-tolerant species whose dominance determines low macrofaunal diversity in the samples) and negative indicator species (sensitive, intolerant species). It has only been applied in the coast of Norway.

2.3.1.8. Benthic Quality Index **(Rosenberg *et al.*, 2004):**

$$BQI = \left(\sum_{i=1}^{n} \left(\frac{A_i}{totA} \times Es50_{0.05i} \right) \right) \times^{10} \log\left(s+1\right)$$

Tolerant species are by definition predominantly found in disturbed environments. That means that they mainly occur at stations with low *ES50*, where *ES* is the diversity value measured by the Hulbert index and *s* de mean number of species. In contrast, sensitive species usually occur in areas with no or minor disturbance, being then associated with high *ES50* values. Taking into account the abundance frequency distribution of a particular species in relation to the *ES50* values at the stations where it has been recorded, the most tolerant individuals of a species are likely to be associated with the lowest *ES50* values. The authors estimated that 5% of the population will be associated to this category, and defined this value as the species tolerance value: $ES50_{0.05}$.

The tolerance value of each species found at a given station is then multiplied by the average relative abundance (***A***) of that species (***i***), in order to weight the common species in relation to the rare ones. Next, the sum is multiplied by the $\log_{10}$ of the mean number of species (***s***) at that station, since higher species diversity is assumed to be related to better environmental quality. All information related to the number of species and their abundance at a given station is therefore used for this quality assessment. This index has only been applied in the Baltic Sea.

2.3.1.9. Conservation Index **(Moreno *et al.*, 2001):**

$$CI = \frac{L}{L + D}$$

Where ***L*** is the proportion of living *Posidonia oceanica* meadow and *D* the proportion of dead meadow coverage.

Different authors applied this index in the neighbourhood of chemical industries, with results leading to establish four grades of *Posidonia* meadow conservation. These grades correspond to increasing impacted areas, allowing the detection of changes in the industry activity as a function of the conservation status in a given location (<0.33: advanced regression; 0.33-0.56: impacted meadow; 0.56-0.79: low to moderate impact; >0.79: high conservation status).

2.3.1.10. Ecological Evaluation Index (Orfanidis *et al.*, 2001):

Shifts in marine ecosystem structure and function are evaluated by classifying marine benthic macroalgae in two ecological groups (ESG I and ESG II). ESG I includes seaweed species with a thick or calcareous thalus, low growth rates and long life cycles, whereas the ESG II includes sheet-like and filamentous seaweeds species with high growth rates and short life cycles.

The absolute abundance (%) of each ESG is estimated by coverage (%) in each sample. It is recommended to obtain at least three samples per season. The estimation of the EEI values and the equivalent ecological status is shown in Table 4.

Table 4

Ecological Evaluation Index values and equivalent ecological status.

Mean coverage of ESG I (%)	Mean coverage of ESG II (%)	ESC	EE	Spatial scale weighted EEI and equivalent ESCs
0 - 30	0 - 30	Moderate	6	≤6 to >4 = Moderate
	>30 - 60	Low	4	≤4 to >2 = Low
	>60	Bad	2	2 = Bad
>30 - 60	0 - 30	Moderate	8	≤8 to >6 = Good
	>30 - 60	Low	6	≤6 to >4 = Moderate
	>60	Bad	4	≤4 to >2 = Low
>60	0 - 30	Moderate	10	≤10 to >8 = High
	>30 - 60	Low	8	≤8 to >6 = Good
	>60	Bad	6	≤6 to >4 = Moderate

B. Bioaccumulator indicator species

There are species classified as bioaccumulative ones, defined as those capable of resisting and accumulating various pollutant substances in their tissues, which facilitates their detection whenever they are in the environment in very low levels, difficult to detect through analytical techniques (Philips, 1977).

The disadvantage of using accumulator indicator species in the detection of pollutants arises from the fact that a number of biotic and abiotic variables may affect the rate at which the pollutant is accumulated, and therefore both laboratory and field tests need to be undertaken so that the effects of extraneous parameters can be identified.

The molluscs group, particularly the bivalves, has been the mostly used to determine the existence and quantity of toxic substances. Individuals of the genera *Mytilus* (De Wolf, 1975; Goldberg *et al.*, 1978; Dabbas *et al.*, 1984; Cossa & Rondeau, 1985; Miller, 1986; Renberg *et al.*, 1986; Carell *et al.*, 1987; Lauenstein *et al.*, 1990; Viarengo & Canesi, 1991; Regoli & Orlando, 1993), *Cerastoderma* (Riisgard *et al.*, 1985; Mohlenberg & Riisgard, 1988; Brock, 1992), *Ostrea* (Lauenstein *et al.*, 1990; Mo & Neilson, 1991) and *Donax* (Marina & Enzo, 1983; Romeo & Gnassia-Barelli, 1988) have been considered ideal in many works to detect the concentration of toxic substances in the environment, due to their sessile nature, wide geographical distribution and capability to accumulate those substances in their tissues and to detoxify when pollution ceases. In that sense, Goldberg *et al.* (1978) introduced the concept of «Mussel Watch» when referring to the use of the molluscs group in the detection of polluting substances. So that, the National Oceanic and Atmospheric Agency (NOAA) in the USA develops since 1980 the «Mussel Watch Program», focused on pollution control lengthways the North American coasts. Programs similar to the North American one exist in Canada (Cossa *et al.*, 1983; Picard-Berube & Cossa, 1983), Denmark (Jensen *et al.*, 1981), Mediterranean Sea (Leonzio *et al.*, 1981; Niencheski, 1982), North Sea

(Golovenko *et al.*, 1981) and in the Australian coasts (Copper *et al.*, 1982; Ritz *et al.*, 1982; Wooton & Lye, 1982; Richardson & Waid, 1983).

Likewise, certain amphipod species are considered capable of accumulating toxic substances (Albrecht *et al.*, 1981; Reish, 1993), as well as polychaete species like *Nereis diversicolor* (Langston *et al.*, 1987; McElroy, 1988), *Neanthes arenaceodentata* (Reish & Gerlinger, 1984), *Glycera alba*, *Tharix marioni* (Gibbs *et al.*, 1983) or *Nephtys hombergi* (Bryan & Gibbs, 1987). Some fish species have also been used in various works focused on the effects of toxic pollution of the marine environment, due to their bioaccumulative capability (Eadie *et al.*, 1982; Gosset *et al.*, 1983; Varanasi *et al.*, 1989) and to the existing relationship between pathologies suffered by benthic fish and the presence of polluting substances (Malins *et al.*, 1984; Couch & Harshbarger, 1985; Myers *et al.*, 1987).

Other authors such as Levine (1984), Maeda & Sakaguchi (1990), Neumann *et al.* (1991) and Storelli & Marcotrigiano (2001) have looked into algae as most favourable for heavy metals, pesticides and radionuclides detection, *Fucus*, *Ascophyllum* and *Enteromorpha* are the most utilised taxa.

2.3.1.11. Ecological Reference Index

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

$$ERI = \frac{measured\ concentration}{BCR}$$

Where *BCR* is the value of the background/reference concentration. The upper limit of *BCR* for hazardous substances in blue mussels according to OSPAR/MON (1998) is provided in Table 5.

Few indices like ERI, based on the use of bioaccumulative species, have been proposed. It is in fact more common the simple measurement of the effects (*e.g.* % incidence, mortality percentage) of a certain pollutant on those species, or the use of biomarkers, which can be useful in evaluating the specificity of responses to natural or anthropogenic changes. Nevertheless, it is very difficult for environmental managers to interpret increasing or decreasing changes in biomarkers data.

Table 5

Upper limits of *BCR* for hazardous substances in blue mussels (OSPAR/MON, 1998)

Substance	Upper limit of BCR value (ng/g dry weight)
Cadmium	550
Mercury	50
Lead	959
Zinc	150000

The Working Group on Biological Effects of Contaminants (WGBEC, 2002) recommended different techniques for biological monitoring programmes, which are summarised in Table 6.

2.3.2. Indices based on ecological strategies

Some indices intend to assess environmental stress effects taking into account the ecological strategies followed by different organisms. That is the case of trophic indices such as the Infaunal Trophic Index proposed by Word (1979) and the Feeding Structure Index (FSI), which are based on organisms' different feeding strategies. Another example is the Nematodes/Copepods Index (Raffaelli & Mason, 1981) which account for the different behaviour of two taxonomic groups under environmental stress situations. Nevertheless, several authors rejected these type of indices due to their dependence of parameters like depth and sediments particle size, as well as because of their unpredictable pattern of variation depending on the type of pollution (Gee *et al.*, 1985; Lambshead & Platt, 1985).

Table 6

Review of different techniques for biological monitoring.

Method	Organism	Issues adressed	Biological significance	Threshold value
Bulky DNA adduct formation	Fish	PAHs, Other synthetic organics	Measures: genotoxic effects. Sensitive indicator of past and present exposure	2 × reference site or 20% change
AChE	Fish and Bivalve molluscs	Organophosphates and carbonates or similar molecules.	Measures: exposure	Minus 2.5 × Reference site
Metallothionein induction	Fish and *Mytilus sp.*	Measures: induction of metallothionein protein by certain metals	Measures: exposure and disturbance of copper and zinc metabolism	2.0 × Reference site
EROD or P4501A induction	Fish	Measures: induction of enzymes with metabolised planar organic contaminants		2.5 × Reference site
ALA-D inhibition	Fish	Lead	Index of exposure	2.0 × Reference site
PAH bile metabolites	Fish	PAHs	Measures: exposure to and metabolism PAHs	2.0 × reference site
Lysosomal stability	Fish and *Mytilus sp.*	Not contaminant specific but responds to a wide variety of xenobiotics, other contaminants & metals	Provides a link between exposure and pathological endpoints	2.5 × Reference site
Lysosomal neutral red retention	*Mytilus sp.*	Not contaminant specific but responds to a wide variety of xenobiotics, other contaminants & metals	Provides a link between exposure and pathological endpoints	2.5 × Reference site

Table 6 *(Continued)*

Review of different techniques for biological monitoring.

Method	Organism	Issues adressed	Biological significance	Threshold value
Early toxicopathic lesions, pre neoplastic and neoplastic liver histopathology	Fish	PAHs	Measures: pathological changes associated with exposure to genotoxic and non-genotoxic carcinogens	2.0 × reference site or 20% change
Scope for growth	Bivalve molluscs	Responds to a wide variety of contaminants	Integrative response which is a sensitive and sublethal measure of energy available for growth	
Shell thickening	*Crassostea gigas*	Specific to organotins	Disruption to pattern of shell growth	
Vitellogenin induction	Male and juvenile fish	Oestrogenic substances	Measures: feminization of male fish and reproductive impairment	
Imposex	Neogastropod molluscs	Specific to organotins	Reproductive interference	2.0 × reference site or 20% change
Intersex	Littorina littorina	Specific to reproductive effects of organotins	Reproductive interference	2.0 × reference site or 20% change
Reproductive success in fish	*Zoarces viviparus*	Not contaminant specific	Measures: reproductive output and survival of eggs and fry in relation to contaminants	

Latter, other proposals appeared, such as the Meiobenthic Pollution Index (Losovskaya, 1983), the Mollusc Mortality Index (Petrov, 1990), the Polychaetes/Amphipods Ratio (Gómez-Gesteira & Dauvin, 2000), or the Index of r/K strategies proposed by De Boer *et al.* (2001), which considers all benthic taxa although emphasising the difficulty of scoring exactly each species through the biological trait analysis.

The R/P Index proposed by Feldman, based on marine vegetation, is highly used in the Mediterranean Sea. It was established as a biogeographical index and accounts for the fact that the number of Rodophyceae species decreases from the Tropics to the Poles. Its application as indicator holds on the higher or lower sensitivity of Phaeophyceae and Rhodophyceae to disturbances. In addition, Belsher (1982) proposed an index based on the qualitative and quantitative dominance of each taxonomic group.

Bellow are listed the indices based on ecological strategies most commonly used in assessing coastal and marine environments.

2.3.2.1. Nematodes/Copepods Index **(Raffaelli & Mason, 1981):**

This index is based on the ratio between the abundances of nematodes and copepods.

$$I = \frac{Nematodes\ abundance}{Copepodes\ abundance}$$

The values of such ratio can increase or decrease in response to higher or lower organic pollution, which expresses a different response of those groups to the input of organic matter into the system. Values over 100 express high organic pollution.

According to different authors, the application of this index should be limited to intertidal areas, since in infralittoral zones, at given depths, despite the absence of pollution, values observed were very high. This fact is explained by the the absence of copepods at such depths, most probaby due to a change in the optimal interstitial habitat for that taxonomic group (Krogh & Spark, 1936 and Wigely & Mcintyre, 1964 in Raffaelli & Mason, 1981).

2.3.2.2. Meiobenthic Pollution Index (Losovskaya, 1983):

$$MPI = \frac{\lg(H{+}1) + \lg(P{+}1)}{2\lg N}$$

Where H, P and N are the numbers (ind m^{-2}) of Harpacticoida, Polychaeta and Nematoda, respectively, in a given benthic sample.

Increasing impacts induce the replacement of harpacticoides and polychaetes by nematodes, and such shift can be traced through changes in the values of the index.

2.3.2.3. Molluscs Mortality Index (Petrov, 1990):

$$MMI\ (\%) = \frac{\textit{Weight of shells of recently dead molluscs}}{\textit{Total weight of living individuals and the shells of molluscs of the same species}}$$

High values of the index are indicative of disturbances.

2.3.2.4. Polychaetes/Amphipods Ratio (Gómez-Gesteira & Dauvin, 2000):

This index follows similar principles to the Nematodes/Copepods Index, but it is applied to the macrofauna level using polychaetes and amphipods. The index was formerly intended to measure the effects of crude pollution.

$$I = Log_{10}\left(\frac{\textit{Polychaetes abundance}}{\textit{Amphipods abundance}} + 1\right)$$

$I \leq 1$: non polluted

$I > 1$: polluted

2.3.2.5. Infaunal Trophic Index (ITI) **(Word, 1980):**

Macrozoobenthic species can be divided in: (1) suspension feeders, which collect detrital materials in overlying water using appendages of the animal or tube or burrow capturing strategies where currents settle these materials adjacent to the organisms); (2) interface feeders, which collect detrital materials that settle on the surface of the sediment - particles that are ingested are generally less than 50 microns in diameter; (3) surface deposit feeders, which collect larger particles that are contained within the upper 2 cm sediments layer, and (4) subsurface deposit feeders, which generally collect particles that are buried deeper than 2 cm). Specialised feeders of this last guild also include species that use methane as a food source. The index value is given by:

$$ITI = 100 - 100/3 \times (0n_1 + 1n_2 + 2n_3 + 3n_4)/(n_1 + n_2 + n_3 + n_4)$$

in which n_1, n_2, n_3 and n_4 are the number of individuals sampled in each of the above mentioned groups.

ITI values near 100 mean that suspension feeders are dominant and that the environment is not disturbed. At values near 0, subsurface feeders are dominant, meaning that the environment is strongly disturbed, probably due to human activities. Index values less than 60 are highly correlated to BOD and TOC or volatile solids in the upper 2 cm of the sediment, while values above 60 are less correlated to accumulation of organic materials in the sediment (Word, 1990).

2.3.2.6. Feeding Structure Index (FSI) **(Milovidova & Alyomov, 1992):**

$$I = \frac{N^o \text{ species of filter} - \text{feeders}}{N^o \text{ species of deposit} - \text{feeders} + \text{predator}}$$

This index is based on the fact that in less eutrophic areas, the number of filter-feeders species is 6 to 8 times greater than in highly eutrophic areas (Petrov & Shadrina, 1996). Like the Word's Infaunal Trophic Index, being based on the nourishing strategy of the different organisms, its application is complex due to the difficulty in assigning correctly a trophic category to each individual.

2.3.2.7. Feldman Index:

$$I = \frac{N^o \text{ of Rhodophyceae} \quad \text{species}}{N^o \text{ of Phaeophyceae} \quad \text{species}}$$

Cormaci & Furnari (1991) detected values over 8 for this index in polluted areas, in Southern Italy, when the normal values in a well balanced community vary between 2.5 and 4.5. Verlaque (1977) studied the effects of a thermal power station, and also found higher values, although this author considered such results due to the presence of communities of warmth affinity. However, Belsher & Boudouresque (1976) analysed the submersed vegetation in small harbours and found out that in such conditions the Phaeophyceae show higher proliferation, which decreases the index value. Therefore, the knowledge about this index behaviour does not seem to be enough to consider it, by itself alone, a good pollution indicator.

2.3.2.8. Belsher Index **(Belsher, 1982):**

$$\text{Qualitative Dominance} = \frac{\%\ \text{species of a taxonomic group}}{\sum \text{population species}} \times 100$$

$$\text{Quantitative Dominance} = \frac{\%\ \text{cover area by a group}}{\text{total cover area}}$$

The ratio between qualitative and quantitative dominance is called tension ψ. Belsher (1982) observed that alongside decreasing pollution gradients, certain groups of algae increase or decrease their tension, establishing the following relation, which was considered a Pollution Index:

$$\frac{\sum \psi i}{\sum \psi j}$$

Where i = groups with decreasing tension and j = groups with increasing tension.

The values of the Pollution Index are high in polluted areas and nearly null in undisturbed zones. This index has only been applied in rocky substrate areas.

2.3.3. Indices based on diversity

Diversity is one of the most used concepts in assessing pollution, based on the fact that the relationship between diversity and environmental disturbances can be seen as an inverse one. Magurran (1989) divides diversity measurements into three main categories:

1. Indices that measure the species ricness, such as the Margalef index, which are essentially measurements of the number of species in a defined sampling unit.
2. Models of species' abundance, as the K-dominance curves (Lambshead *et al.*, 1983) or the log normal model (Gray, 1979), which describe the distribution of their abundance, going from those that represent situations in which there is a high uniformity, to those that characterise cases in which the abundance of each species is very unequal. It must be said that the lognormal model deviation was rejected by several authors since it was impossible to find any benthic marine sample that clearly responded to such distribution model (Shaw *et al.*, 1983; Hughes, 1984; Lambshead & Platt, 1985).

3. Indices based on the proportional abundance of different species, which intend to account for richness and uniformity in a simple expression. This category of indices can also be divided into those based on a) statistics; b) Information Theory, and c) species dominance. Indices derived form the Information Theory, such as the Shannon-Wiener, are based on something logical: 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 Simpson or Berger-Parker ones are referred as measurements that ponder the abundance of the mostly common species, instead of species richness.

In the meantime, Average Taxonomic Diversity and Distinctness measures have been proposed and used by some researchers (*e.g*. Warwick & Clarke, 1995, 1998; Clarke & Warwick, 1999) to evaluate biodiversity in the marine environment, taking into account taxonomical, numerical, ecological, genetical and filogenetical aspects of diversity. These measures address some of the problems identified in relation to species richness and other diversity indices (Warwick & Clarke, 1995).

The most commonly used diversity measures are listed below.

2.3.3.1. Shannon-Wiener Index **(Shannon & Weaver, 1963):**

This index is based on the Information Theory. It assumes that individuals are sampled at random, out of an «indefinitely large» community, and that all the species are represented in the sample and can be estimated according to the algorithm:

$$H' = -\sum p_i \log_2 p_i$$

Where p_i is the proportion of individuals belonging to species i in the sample. The real value of p_i is unknown, but it is estimated through the ratio N_i/N, where N_i =number of individuals of the species i and N = total number of individuals.

The units for the index depend on the log used. So, for $\log_2$, the unit is bits/individual; «natural bels» and «nat» for $\log_e$; and «decimal digits» and «decits» for $\log_{10}$.

The index can usually take values between 0 and 5, and maximal values above 5 bits/individual are very rare. In this case, diversity is a logarithmic measurement showing, to a certain extent, asintonic character, which makes it a little sensitive index in the range of values next to the upper limit (Margalef, 1978).

As an ordinary basis, in the literature, index low values are considered an indication of pollution (Stirn *et al.*, 1971; Anger, 1975; Hong, 1983; Zabala *et al.*, 1983; Encalada & Millan, 1990; Calderón-Aguilera, 1992; Pocklington *et al.*, 1994; Engle *et al.*, 1994, Mendez-Ubach, 1997; Yokoyama, 1997). But one of the problems arising with its use is the lack of objectivity when trying to establish in a precise manner from what threshold should one start taking into account the index values an indicating the effects of such pollution.

For instance, Molvaer *et al.* (1997) established the following relation between the Shannon-Wiener index values and the different levels of ecological quality (Table 7), in accordance to what is recommended by the European Water Framework Directive (EC, 2000).

Table 7

Categories considered as a function of Shannon-Wiener index values, according to Molvaer *et al.* (1997).

Classification	Shannon-Wiener value
High Status	>4 bits/indv
Good Status	4 - 3 bits/indv
Moderate Status	3 - 2 bits/indv
Poor Status	2 - 1 bits/indv
Bad Status	1 - 0 bits/indv

Detractors of this index based their criticisms on its lack of sensitivity when it comes to detecting the initial stages of pollution (Leppäkoski, 1975; Pearson & Rosenberg, 1978; Rygg, 1985). For instance, Gray (1979), studying the effects of a cellulose paste factory waste, set out the uselessness of this index as it responses to such obvious changes that there is no need of a tool to detect them.

Ros & Cardell (1991), in their study about the effects of great industrial and human domestic pollution, consider the index as a partial approach to the knowledge of pollution effects on marine benthic communities and, without any further explanation to that statement, set out a new structural index proposal, which lack of applicability has already been shown in Salas (2002).

2.3.3.2. Pielou Evenness Index **(Pielou, 1969)**:

$$J' = H'/H'_{max} = H'/\log S$$

Where H'_{max} is the maximum possible value of Shannon diversity.

The values of this index may vary from 0 to 1.

2.3.3.3. Margalef Index

The Margalef index quantifies diversity by relating specific richness to the total number of individuals.

$$D = (S\text{-}1)/\log_2 N$$

For S = number of species and N = total number of individuals.

The author did not establish any reference values, and in fact the main problem when applying this index is the absence of a limit value, and therefore the difficulty in establishing such reference values. Ros & Cardell (1991) consider values below 4 as typical of polluted areas. On the other hand, Bellan-Santini (1980) settled a different limit, considering a polluted area when the index takes values below 2.05.

2.3.3.4. Berger-Parker Index

This index expresses the proportional importance of the most abundant species, and may be computed using the following algorithm:

$$D = n_{max}/N$$

Where n_{max} is the number of individuals of the one most abundant species and N is the total number of individuals. The index values may vary from 0 to 1 and, contrarily to other diversity indices, higher values correspond to a lower diversity.

2.3.3.5. Simpson Index **(Simpson, 1949):**

Simpson (1949) proposed a diversity index which accounts for the probability that two whatever individuals randomly sampled from an infinitely large community could belong to the same one species:

$$D = \sum p_i^2$$

Where p_i is the proportion of individuals from species i in the community. To calculate the index for a finite community the following algorithm can be used:

$$D = \sum [n_i (n_i\text{-}1)/N (N\text{-}1)]$$

Where n_i is the number of individuals of species i and N is the total number of individuals.

Likewise the Berger-Parker Index, the Simpson Index may vary from 0 to 1, it has no dimensions and, in the same way, higher values correspond to lower diversity.

2.3.3.6. Hulbert Index (Hulbert, 1971):

$$ESn = \sum_{i=1}^{n} \left[1 - \frac{(N - Ni)!\,(N - n)}{(N - Ni - n)\,!N!} \right]$$

Where N is the total number of individuals in a sample and Ni is the number of individuals of the i-th species.

The idea is to generate an absolute measure of species richness, which can be compared across samples of very differing sizes. Nevertheless, the validity of this index depends on the assumption that the individuals of each species are randomly distributed, which is not always the case.

2.3.3.7. Fisher's α *Index* (Fisher *et al.*, 1943):

$$S = \alpha \times \ln(1 + \frac{n}{\alpha})$$

Where S is the number of taxa, n is the number of individuals and α is the Fisher's α, which is the shape parameter, fitted by maximum likelihood, under the assumption that the species abundance distribution follows a log series. This has certainly been shown to be the case for some ecological data sets, but can by no means be universally assumed, and its use is restricted to genuine (integral) counts (Warwick & Clarke, 2001).

2.3.3.8. Rarefaction Curves (Sanders, 1968):

Rarefaction curves are plots of the number of individuals on the x-axis against the number of species on the y-axis. The more diverse the community is, the steeper and more elevated the rarefaction curve is.

2.3.3.9. Deviation from the log normal distribution **(Gray & Mirza, 1979):**

This method, proposed by Gray & Mirza (1979), is based on the assumption that when a sample is taken from a community, the individuals' distribution tends to follow a log-normal model. The adjustment to a logarithmic normal distribution assumes that the population is ruled by a certain number of factors, being at a steady equilibrium. Consequently, any deviation from such distribution implies that some perturbation is affecting it.

2.3.3.10. Ranked Species Abundance (Dominance) Curves:

It consists on ranking the species (or higher taxa) in decreasing order of their importance in terms of abundance or biomass. The ranked abundances, expressed as a percentage of the total abundance of all species, are plotted against the relevant species rank.

2.3.3.11. K-Dominance Curves **(Lambshead *et al.*, 1983)***:*

The K-Dominance Curve is the representation of the accumulated percentage of abundance versus the logarithm of the sequence of species ranked in a decreasing order The slope of the straight line obtained allows the valuation of the pollution grade. The higher the slope is, the higher the diversity is, as well.

2.3.3.12. Average Taxonomic Diversity and Distinctness measures **(Warwick & Clarke, 1995)***:*

Warwick & Clarke (1995) proposed several measures which integrate information usually provided by species richness and other diversity indices. Such measures are based on the different species abundances (denoted by x_i, the number of individuals of species *i* in the sample) and on the taxonomic distance (ω_{ij}) through the classification tree between every pair of individuals (the first from species i and the second from species j). The measures proposed are the following ones:

a) Taxonomic Diversity (Δ)

It consists of the average taxonomic distance apart of every pair of individuals in the sample or, the expected path length between any two individuals chosen at random.

$$\Delta = \left[\sum\sum_{i<j} \omega_{ij} x_i x_j\right] / \left[n(n-1)/2\right]$$

Where the double summation is over all pairs of species i and j ($i,j=1,2,...,S; i<j$), and $n = \sum_i x_i$, the total number of individuals in the sample.

b) Average Taxonomic Distinctness (Warwick & Clarke, 1995):

To remove the dominating effect of the species abundances distribution, Warwick & Clarke (1995) proposed to divide the Average Taxonomic Diversity Index by the Simpson Index (Simpson, 1949), giving the Average Taxonomic Distinctness index.

$$\Delta^* = \left[\sum\sum_{i<j} \omega_{ij} x_i x_j\right] / \left[\sum\sum_{i<j} x_i x_j\right]$$

When quantitative data are not available and samples provided simple lists of species (presence/absence data), the Average Taxonomic Distinctness takes the following form:

$$\Delta^+ = \left[\sum\sum_{i<j} \omega_{ij}\right] / \left[s(s-1)2\right]$$

Where s, is the observed number of species in the sample and the double summation ranges over all pairs i and j of the species ($i<j$).

c) Total Taxonomic Distinctness (TTD):

The TTD was proposed by Clarke & Warwick (1995) as a useful measure of total taxonomic breadth of an assemblage, as a modification of species richness, which allows for the species inter-relatedness.

$$s\Delta^{+} = \sum_{i}\left[\left(\sum_{i\neq j}\omega_{ij}\right)/\left(s-1\right)\right]$$

This measure is the average taxonomic distance from species i to every other species, summed over all species, i=1,2,..s.

d) Variation in Taxonomic Distinctness (STTD):

This measure was proposed in order to account for the «evenness» of the different *taxa* distribution across the hierarchical taxonomic tree.

$$\Lambda^{+} = \left[\sum\sum_{i\neq j}\left(\omega_{ij}-\bar{\omega}\right)^{2}\right]/\left[s\left(s-1\right)\right]$$

Clarke & Warwick (2001) have shown that the Variation in Taxonomic Distinctness has the same desirables sampling properties as Average Taxonomic Distinctness, namely a lack of dependence of its mean value on the sample size.

To estimate Taxonomic Diversity indices, a hierarchical Linnean classification is used as a proxy for cladograms representing the relatedness of individual species. For each location, a composite taxonomy is compiled and five taxonomic levels are considered (species, genus, family, order, class and phylum). Generally, these diversity indices are calculated from fauna abundances using PRIMER 5 (Software package from Plymouth Marine Laboratory, UK).

2.3.4. Indicators based on species biomass and abundance

Other approaches account for the variation of organism's biomass and abundance as a measure of environmental disturbances. These approaches encompass methods such as SAB Curves (Pearson & Rosenberg, 1978), consisting of a comparison between the curves resulting from ranking the species as a function of their representativeness in terms of both their abundance and biomass. The use of this method is not advisable because it is purely graphical, which leads to a high degree of subjectivity and does not allow to relate it quantitatively with the environmental factors. In his turn, the ABC Method (Warwick, 1986) also involves the comparison between the cumulative curves of species biomass and abundance, from which Warwick & Clarke (1994) derived the W-Statistic Index.

2.3.4.1. ABC Method (Warwick, 1986):

This method is based on the assumption that, for a given community, the distribution of the number of individuals and the biomass from each species do not show the same variation pattern. It consists, in fact, of an adaptation from the already mentioned K-dominance curves, although showing in a single graphic the K-dominance and the biomass curves. The graphics allow ploting the interval of species (in the abscissa axis), arranged in decreasing order according to a logarithmic scale, against the cumulative dominance curves (in the ordinate axis).

Three different situations can occur as function of the disturbance's degree affecting the community (Figure 1):

1. In a non disturbed system, a fairly low number of relatively large individuals from few species will contribute with most of the biomass, and at the same time, the individuals' distribution among the different species

is more equitative. Graphically, the biomass curve will be plotted above the abundance one, indicating higher numeric diversity than biomass diversity.

2. In communities under moderate disturbance conditions, the biomass cumulative curve will not show such an important contribution of just a few species represented by a low number of individuals as in the previous case, but on the other hand abundances increase. Graphically, the biomass and abundance curves come out intersected.

3. In the case of communities under intense disturbances, a few species only will represent most of the individuals, all of a small size, which explains why the biomass from each one of the species is low and more equitatively shared. Graphically, the abundance curve come out above the biomass curve, indicating higher biomass than numerical diversity in the distributions.

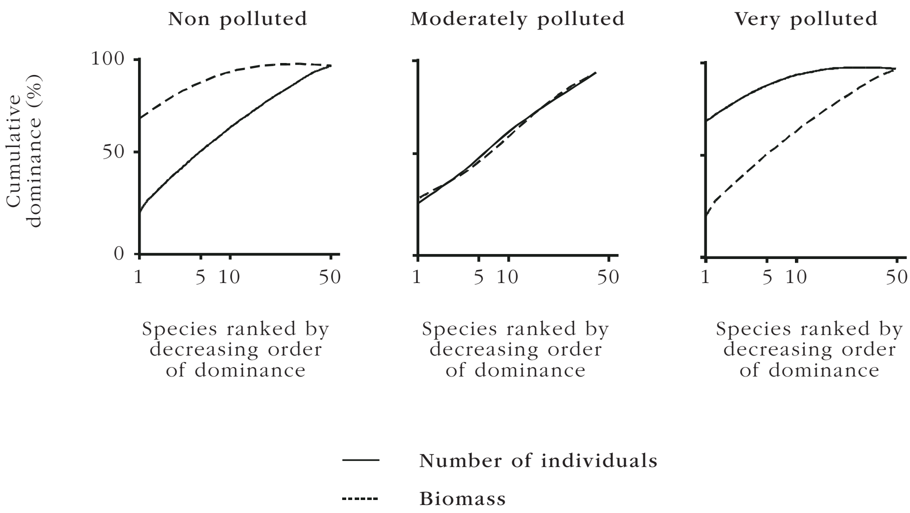

Figure 1. Hypothetical K-dominance curves for species biomass and number, showing unpolluted, moderately polluted, and heavily polluted conditions On the horizontal (X) axis, the species are ranked by decreasing order of importance, using a log scale. On the vertical (Y) axis, the percentage dominance is plotted using a cumulative percentage scale (Warwick, 1986).

2.3.4.2. W-Statistic Index **(Warwick & Clarke,1994):**

Some authors like Beukema (1988), Clarke (1990), McManus & Pauly (1990), Meire & Dereu (1990) have tried to convert the ABC method into a measurable index. Clarke's (1990) approach became the most commonly accepted.

$$W = \sum_{i=1}^{s} (B_i - A_i) \Big/ 50(S-1)$$

Where B_i is the biomass of species i, A_i the abundance of specie i, and S is the number of species. The index can take values from +1, indicating a non-disturbed system (high status) to -1, which defines a polluted situation (bad status). Values close to 0 indicate moderate pollution (moderate status).

This approach is specific for organic pollution and has been applied, with satisfactory results, to soft bottom tropical communities (Anderlini & Wear, 1992; Agard *et al.*, 1993), to experiments (Gray *et al.*, 1988), to fish factoring disturbed areas (Ritz *et al.*, 1989), and on coastal lagoons (Reizopoulou *et al.*, 1996, Salas, 2002). However, Ibanez & Dauvin (1988), Beukema (1988), Weston (1990), Craeymeersch (1991) and Salas *et al.* (2004) obtained confusing results after applying this method to assess the environmental status in estuarine zones, which was induced by the appearance of dominant species in normal conditions, favoured not by organic pollution but by other environmental factors. On the other hand, in spite of having been designed to be applied to benthic macrofauna, Abou-Aisha *et al.* (1995) applied this method in three areas of the Red Sea to detect the impact of phosphorus wastes on macroalgae. Nevertheless, problems may arise when applying it to marine vegetation, due to obvious difficulties in counting the number of individuals from vegetal species.

2.3.5. Indicators integrating all environment information

From a more holistic point of view, some authors proposed indices capable of integrating the whole environmental information. A first approach was developed by Satsmadjis (1982) for application in coastal areas, relating sediment particles size to benthic organisms diversity. Wollenweider *et al.* (1998) developed a Trophic Index (TRIX) integrating chlorophyll *a*, oxygen saturation, total nitrogen and phosphorus to characterise the trophic state of coastal waters. In the same way, Fano *et al.* (2003) proposed the Ecological Functional Index that has in account the macrofaunal and macrophytes abundance/biomass. In a progressively more complex way, other indices such as the Index of Biotic Integrity (IBI) for coastal systems (Nelson, 1990), the Benthic Index of Environmental Condition (Engle *et al.*, 1994; Macauley *et al.*, 1999), or the Chesapeake Bay B-BI Index (Weisberg et al, 1997) and the Carolina Province B-IBI (Van Dolah *et al.*, 1999) include physicochemical factors, diversity measures, specific richness, taxonomical composition, and the system's trophic structure.

Similarly, a set of specific indices of fish communities has been developed to measure the ecological status of estuarine areas. The Estuarine Biological Health Index (BHI) (McGinty & Lider, 1997) combines two separate measures (health and importance) into a single index. The Estuarine Fish Health Index (FHI) (Cooper *et al.*, 1993) is based on both qualitative and quantitative comparisons with a reference fish community. The Estuarine Biotic Integrity Index (EBI) (Deegan *et al.*, 1993) reflects the relationship between anthropogenic alterations in the ecosystem and the status of higher trophic levels, 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.

Nevertheless, these indicators are rarely used in a generalised way because they have usually been developed to be applied in a particular system or area, which turns them dependent on the type of habitat and seasonality. On the other hand, they are difficult to apply as they require a large amount of data of different nature.

2.3.5.1. Coefficient of Pollution **(Satsmadjis, 1985):**

The estimation of this index is based on several integrated equations. These equations are:

$$S'=s+t/(5+0.2s)$$
$$i_0=(-0.0187s'2+2.63s'-4)(2.20-0.0166h)$$
$$g'=i/(0.0124i+1.63)$$
$$P=g'/[g(i/i_0)^{1/2}]$$

Where: P = coefficient of pollution; S' = sand equivalent; S = percent sand; t = percent silt; i_0 = theoretical number of individuals; i = number of individuals; h = station depth; g' = theoretical number of species and g = number of species.

2.3.5.2. Benthic Index of Environmental Condition **(Engle *et al.*, 1994):**

Benthic Index of Environmental Condition = (2.3841 × proportion of expected diversity) + (-1.6728 × proportion of total abundance of tubifids) + (0.6683 × proportion of total abundance of bivalves)

The expected diversity is calculated throughout Shannon-Wiener index adjusted for salinity.

Expected Diversity $= 0.75411 + (0.00078 \times salinity) + (0.00157 \times salinity^2) + (-0.00030 \times salinity^3)$

This index was developed for estuarine macrobenthos in the Gulf of Mexico in order to discriminate between areas with degraded environmental conditions and areas with non-degraded or reference conditions. Its final development step involved the estimation of discriminating scores for all samples sites and normalising calculated scores to a scale of 0 to 10, setting the break point between degraded and non-degraded reference sites at 4.1. Therefore, values lower than 4.1 indicate degraded conditions, values higher than 6.1 indicate non-degraded situations, and values between 6.1 and 4.1 reveal moderate disturbance.

2.3.5.3. Trophic Index **(TRIX) (Wollenweider *et al.*, 1998):**

The index integrates in a single value parameters like chlorophyll a, oxygen saturation, total nitrogen and phosphorus concentration.

$$TRIX = \frac{k}{n} \times \sum (Mi - Li) / (Ui - Li)$$

In which k=10 (scaling the result between 0 and 10), n=4 (number of variables integrated, M_i = measured value of variable i, U_i = upper limit of variable i, L_i = lower limit of value i.

The resulting TRIX values are dependent on the upper and the lower limits chosen and indicate how close the current state of a system is to the natural state. However, the comparison of TRIX values obtained for different areas becomes more difficult. In general, when a wide, more general range is used for the limits, TRIX values for different areas are more easily compared to each other.

2.3.5.4. Ecological Functional Index **(EQI) (Fano *et al.*, 2003):**

This index is based on the characteristics of the primary producers and benthic faunal communities and has been designed to assess the ecological quality of the coastal lagoons. The following parameters are taken into account: macrofaunal abundance; number of taxa; taxonomic diversity; functional diversity; macrofaunal biomass; phytoplankton biomass and macrophytal biomass. Each one of these attributes, which are expressed by heterogeneous units, is then transformed into a dimensionless quality scale ranging from 0 to 100, simply by assigning 100 to the highest value, and by normalising to 100 all the other values. Once all attributes are expressed by means of this scale, they are combined to obtain the integrated index, whose maximum theoretical value will vary from 700 to 800, depending on whether macroalgae are present in a particular habitat. These values would correspond to the optimum condition of the index, irrespective of the units and magnitudes used to measure the different individual attributes. Obviously, the closer the actual values are to, let us say, 800, the better the condition of the environment.

EQI also allows comparisons between sites from different lagoons (nEQI). Data sets from the different lagoons are merged into a worksheet so that the value of each attribute can be rescaled, using the same quality scale of 0 to 100 on the complete data set. Finally, scores are summed and divided by the number of attributes measured in each different lagoon. In this way, the use of EQI can derive a series of continuous values, from 0 to 800 (nEQI: from 0 to 100). The result obtained is a functional classification of the sites within a lagoon or between different lagoons.

Up to now, this index only has been applied in three coastal lagoons in Italy (Sacca di Goro, Valle Fattibello and Valli di Comacchio).

2.3.5.5. B-IBI **(Weisberg *et al.*, 1997):**

Ten metrics are used to estimate the B-IBI values (Weisberg *et al.*, 1997): Shannon-Wiener index; total species abundance; total species biomass; percent abundance of pollution-indicative taxa; percent abundance of pollution-sensitive taxa; percent biomass of pollution-indicative taxa; percent biomass of pollution-sensitive taxa; percent abundance of carnivore and omnivores; percent abundance of deep-deposit feeders.

The scoring of metrics to estimate B-IBI is carried out by comparing the value of a metric from a given sample of unknown sediment quality to thresholds established from reference data distributions (Table 8). This index was developed to establish ecologic status of Chesapeake Bay, and therefore it is habitat type and season specific, being advisable to use in spring only.

Table 8

Thresholds used to score each metric of the B-IBI.

	SCORING CRITERIA		
	5	**3**	**1**
Tidal Freshwater			
Shannon-Wiener	≥1.8	1-1.8	<1
Abundance (m^{-2})	≥1000-4000	500-1000 or ≥4000-10000	<500 or ≥10000
Biomass (gm^{-2})	≥0.5-3	0.25-0,5 or ≥3-50	<0.25 or ≥50
Abundance pollution indicative taxa (%)	≤25	25-75	>75
Oligohaline			
Shannon-Wiener	≥2.5	1.9-2.5	<1.9
Abundance (m^{-2})	≥1500-3000	500-1500 or ≥3000-8000	<500 or ≥8000
Biomass (gm^{-2})	≥3-25	0.5-3 or ≥25-60	<0.5 or ≥60
Abundance pollution indicative taxa (%)	≤25	25-75	>75
Abundance sensitive taxa (%)	≥40	10-40	<10
Low Mesohaline			
Shannon-Wiener	≥2.5	1.7-2.5	<1.7
Abundance (m^{-2})	≥1500-2500	500-1500 or ≥2500-6000	<500 or ≥6000
Biomass (gm^{-2})	≥5-10	1-5 or ≥10-30	<1 or ≥30
Abundance pollution indicative taxa (%)	≤10	10-20	>20
Biomass pollution sensitive taxa (%)	>80	40-80	<40
Biomass >5cm below sediment-water interface (%)	≥80	10-80	<10
High Mesohaline Sand			
Shannon-Wiener	≥3.2	2.5-3.2	<2.5
Abundance (m^{-2})	≥1500-3000	1000-1500 or ≥3000-5000	<1000 or ≥5000
Biomass (gm^{-2})	≥3-15	1-3 or ≥15-50	<1 or 50
Abundance pollution indicative taxa (%)	<10	10-25	>25
Abundance sensitive taxa (%)	≥40	10-40	<10
Abundance carnivores & omnivores (%)	≥35	20-35	<20

Table 8 *(Continued)*

Thresholds used to score each metric of the B-IBI.

	SCORING CRITERIA		
	5	**3**	**1**
High Mesohaline Mud			
Shannon-Wiener	≥3	2-3	<2
Abundance (m^{-2})	≥1500-3000	1000-1500 or ≥2500-5000	<1000 or ≥5000
Biomass (gm^{-2})	≥2-10	0.5-2 or ≥10-50	<1000 or ≥5000
Biomass pollution indicative taxa (%)	≤5	5-30	>30
Biomass pollution-sensitive taxa (%)	≥60	30-60	<30
Abundance carnivores & omnivores (%)	<25	10-25	<10
Biomass >5 cm below sediment-water interface (%)	≥60	10-60	<10
Polyhaline Sand			
Shannon-Wiener	≥3-5	2.7-3.5	<2.7
Abundance (m^{-2})	≥3000-5000	1500-3000 or ≥5000-8000	<1500 or ≥8000
Biomass (gm^{-2})	≥5-20	1-5 or ≥20-50	<1 or ≥50
Abundance pollution indicative taxa (%)	≤5	5-15	>15
Biomass pollution-sensitive taxa (%)	≥50	25-50	<25
Abundance deep-deposit feeders (%)	>25	10-25	<25
Polyhaline Mud			
Shannon-Wiener	>3.3	2.4-3.3	<2.4
Abundance (m^{-2})	≥1500-3000	1000-1500 or ≥3000-8000	<1000 or ≥8000
Biomass (gm^{-2})	≥3-10	0.5-3 or ≥10-30	<0.5 or ≥30
Biomass pollution indicative taxa (%)	≤5	5-20	>20
Biomass pollution-sensitive taxa (%)	≥60	30-60	<30
Abundance carnivores & omnivores (%)	≥40	25-40	<25
Taxa > 5cm below sediment--water interface (%)	≥40	10-40	<10

2.3.5.6. B-IBI for Carolina Province **(Van Dolah *et al.*, 1999):**

This index is a modification of the B-IBI developed for the Chesapeake Bay (Weisberg *et al.*, 1997) and is calculated using the average score of the following metrics: mean abundance; mean number of taxa; percentage of abundance of the top two numerical dominants and percentage abundance of pollution sensitive taxa.

2.3.5.7. Biotic Integrity (IBI) for fishes **(McGinty & Lider, 1997):**

A fish based Index of Biotic Integrity (IBI) was developed for tidal fish communities of several small tributaries of the Chesapeake Bay (Jordan *et al.*, 1990, Vaas & Jordan, 1990, Carmichael *et al.*, 1992).

Nine metrics are used to calculate the index having in account species richness, trophic structure and abundance: number of species; number of species comprising 90% of the catch; number of species in the bottom trawl; proportion of carnivores; proportion of planktivores; proportion of benthivores; number of estuarine fish; number of anadromous fish and total fish with Atlantic menhaden removed.

The quantification of the different metrics utilised to estimate the index is carried out by comparing the value of a metric from the sample of unknown water quality to thresholds established from reference data distributions.

2.3.5.8. Fish Health Index **(FHI) (Cooper *et al.*, 1993):**

This index is based on the Community Degradation Index (CDI) developed by Ramm (1988, 1990) which measures the degree of dissimilarity (degradation) between a potential fish assemblage and the actual measured fish assemblage.

The FHI provides a measure of the similarity (health) between the potential and actual fish assemblages and is calculated using the formula:

$$FHI = 10\,(J)[\mathrm{Ln}\,(P)/\mathrm{Ln}\,(P_{max})]$$

Where J = the number of species in the system divided by the number of species in the reference community; P = the potential species richness (number of species) of each reference community and $Pmax$ = the maximum potential species richness from all the reference communities. The index ranges from 0 (poor) to 10 (good).

The FHI was used to assess the state of South Africa's estuaries (Cooper *et al.*, 1993; Harrison *et al.*, 1994, 1995, 1997, 1999). Although the index has proved to be a useful tool in condensing information on estuarine fish assemblages into a single numerical value, the index is based only on presence/absence data, and consequently does not take into account the relative proportions of the various species present.

2.3.5.9. Estuarine Ecological Index **(EBI) (Deegan *et al.*, 1993):**

The EBI includes the following eight metrics: total number of species; dominance; fish abundance; number of nursery; number of estuarine spawning species; number of resident species; proportion of benthic associated species; proportion of abnormal or diseased fishes.

The usefulness of this index requires that it reflects not only the current status of fish communities but also its applicability over a wide range of estuaries, although this is not entirely achieved (Bettencourt *et al.*, 2004).

2.3.6. 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 evelopment, and b) to act as orientors (goal functions) in models development, as already referred above. Such orientors intend to account for suitable system-oriented characteristics, expressing natural tendencies of ecosystems development (Marques *et al.*, 1998).

In general, these 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 (Jørgensen & Marques, 2001). That is for instance the case of Ascendency (Ulanowicz, 1980, 1986; Ulanowicz & Norden, 1990), and Exergy (Jørgensen & Mejer, 1979; 1981), a concept derived from thermodynamics, which can be seen as energy with a build in measure of quality, which have been tested in several studies (*e.g.* Nielsen, 1990; Jørgensen, 1994, Fuliu, 1997, Marques *et al.*, 1997; 2003).

2.3.6.1. Eco-Exergy **(Jørgensen & Mejer, 1979; 1981):**

$$Ex = RT \times \sum C_i \times \beta_i$$

Where R is the gas constant, T is the absolute temperature, C_i is the concentration in the ecosystem of component i (*e.g.* biomass of a given taxonomic group or functional group), β_i is a factor able to express roughly the quantity of information embedded in the organisms organisms biomass, namely accounting for the genome size (Table 9). Detritus was chosen as reference level, *i.e.* $\beta_i = 1$ and Exergy in biomass of different types of organisms is expressed in detritus energy equivalents.

If the total biomass in the system remains constant then Exergy variations will rely upon its structural complexity. Specific Exergy is defined as Exergy/biomass. Both Exergy and Specific Exergy may be used as indicators in environmental management, being advisable to apply them complementary (Marques *et al.*, 1997).

This formulation of Exergy, referred in first place as Modern Exergy (Jørgensen *et al.*, 1995) does not correspond to the strict thermodynamic definition of the concept, but provides nevertheless an approximation of Exergy values. In this sense it was proposed to call it Exergy Index (Marques *et al.*, 1997; 1998), or Ecological Exergy (Fonseca *et al.*, 2000; 2002), a term finally adopted by Jørgensen as Eco-Exergy (see for instance Jørgensen *et al.*, 2005). This formulation allows to empirically estimate the Exergy Index from normal sets of ecological data, *e.g.* organism's biomass, provided that βi value for the different types of organisms is known.

Marques *et al.* (1997) suggested the use of nuclear DNA (C-values) content to evaluate the parameter β, assuming the DNA content as a measure of the information carried in its genome, acquired throughout the evolutionary process. On the other hand, Fonseca *et al.* (2000; 2002) in accordance with the studies of Lewin (1994) claim that similar organisms in complexity may have significantly different nuclear DNA content and at higher evolutionary levels, genome size losses correspondence to the increase in structural complexity of organisms due to the presence of repetitive DNA sequences. Thus, non-repetitive DNA content, rather than the total genome should better evaluates organism complexity. Therefore, it could be assumed that to each adjacent triplet of nucleotides from non-repetitive DNA corresponds a transcribed RNA-signal (from regulatory genes or structural genes). Hence, the non-repetitive DNA could be considered as an approximate estimation (although rough) of the overall «coding capacity» of the genome and used in the evaluation of the parameter β. For this reason, Fonseca *et al.* (2000) propose that, instead of C-values to estimate weighing factors β for each species, the lowest (known) C-value in different groups of organisms is preferable. Anyway, the estimation of correct β values constitutes one of the major difficulties involved in applying the Exergy concept in Ecology, and requires further research (Jørgensen *et al.*, 2005).

The minimum DNA contents (lowest C-values) of several groups used in the estimation of the β parameter are given in Table 9. Thermodynamically speaking, Exergy applied in Ecology is a measure of the distance between a given state of an ecosystem and what the system would be at thermodynamic equilibrium (Jørgensen & Mejer, 1979). In other words, the Exergy of an ecosystem at thermodynamic equilibrium would be zero. This means that, during ecological succession, Exergy is used to build up biomass, which in turn stores Exergy, and therefore Exergy represents a measure of the biomass structure plus the information embedded in the biomass (Jørgensen *et al.*, 2002).

In a trophic network, biomass and Exergy will flow between ecosystem compartments, supporting different processes by which Exergy is both degraded (respiration) and stored (growth production) in different forms of biomass belonging to different trophic levels. More complex organisms have more built in information and are further away from thermodynamic equilibrium than simpler organisms. Therefore, more complex organisms have also more built in Exergy (thermodynamic information) in their biomass than the simpler ones. On the other hand, ecological succession drives from more simple to more complex ecosystems, which seem at a given point to reach a sort of balance between keeping a given structure, emerging for the optimal use of the available resources, and modifying the structure, adapting it to a permanently changing environment.

Exergy has been considered as a promising indicator of ecosystem integrity by several authors (Nielsen, 1990; Jørgensen, 1994; Fuliu, 1997), acquiring a considerable interest in the context of systems ecology. Actually, Exergy has been applied as indicator of the state of ecosystems in a number of European lakes, mainly through the studies of Jørgensen (1994), and Nielsen (1992, 1994). The lakes have been investigated in connection with natural or human induced changes of the lake ecosystems, such as eutrophication and biomanipulation. In addition, four other works investigated the relations between Exergy based indices and biodiversity in a freshwater system, an estuary, a coastal lagoon and an intertidal rocky shore, respectively (Jørgensen & Padisak, 1996; Marques *et al.*, 1997; Salas *et al.*, 2005; Patrício *et al.*, 2006). Results showed that the Exergy based indices appeared to be able to provide useful information regarding the state of the systems.

Table 9

Values for the number of genes and cell types and for the weighting factor (β) to estimate Exergy. Values of weighting factors are based on the number of information genes. The Exergy content of the organic matter in the various organisms is compared with Exergy contained in detritus. Estimations were carried out according to the method described by Jørgensen *et al.* (1995), based on analytical work (Fonseca *et al.*, 2000) and on literature sources (Lewin, 1994).

Organisms	10^{-12} g DNA/cell	Number of genes	Number of cell types	Weighting factor
Detritus	0	0	0	1
Bacteria	0.005	600	1-2	3
Algae	0.009	850	6-8	3.9
Fungus	0.03	3000	6-7	10.2
Insects	—	—	—	70
Crustaceans	—	—	—	230
Annelids worms	20	100000	60	50
Molluscs	—	—	—	280
Gastropods	—	—	—	450
Bivalves	—	—	—	760
Echinoderms	—	—	—	260
Fish	20	100000-120000	70	287-344
Birds	—	120000	—	1100
Amphibians	—	120000	—	800
Reptiles	—	120000	—	1100
Mammals	50	140000	100	2000
Human	90	250000	254	1300

2.3.6.2. Ascendency **(Ulanowicz, 1980):**

The emphasis in ecology has been shifting toward a vision of the ecosystem as a system of interactions (Fasham, 1984; Frontier & Pichod-Viale, 1995), meaning the center of interest has become less the state of the biomass of the different groups of organisms, than the status of the interactions between them, as quantified by flows of matter or energy (Niquil, 1999).

Any index used in such attempts must combine the attributes of system activity level and community structure. One of such measures derives from the analysis of networks of trophic exchanges and is called the system «ascendency». Ulanowicz (1980) defines ascendency as an index that quantifies both the level of system activity and the degree of its organization whereby it processes material in autocatalytic fashion.

Ascendency is a rather abstract concept that nevertheless reveals manifold attributes when viewed from a variety of aspects. Ascendency was originally created to quantify the developmental status of an ecosystem. If one suspects that a particular disturbance has negatively impacted the ecosystem, ascendency can be invoked to test that hypothesis quantitatively, provided sufficient data are available to construct networks of exchanges before and after the impact. Not only can one make before and after comparisons, but the developmental stages of disparate ecosystems can also be compared with one another (*e.g.* Ulanowicz & Wulff, 1991).

Using ascendency, it is possible to determine quantitatively whether a system has grown or shrunk, developed or regressed. Furthermore, particular patterns of changes in the information variables can be used to identify processes that hitherto had been described only verbally (Ulanowicz, 2000). For example, eutrophication can be described in terms of network attributes as any increase in system ascendency (due to a nutrient enrichment) that causes a rise in total system throughput that more than compensates for a concomitant fall in the mutual information (Ulanowicz, 1986). This particular combination of changes in variables allows one to distinguish between instances of simple enrichment and cases of undesirable eutrophication.

Estimating a system's ascendancy implies the calculation of a set of information indices, followed by trophic and cycling analyses.

a) Information indices

Total System Throughput (TST): The differences in system activity are gauged by the relative values of the TST. The total system throughput is simply the sum of all transfer processes occurring in the system. That is $TST = \sum_{p,q} T_{pq}$ for all possible transfers $\boldsymbol{T_{pq}}$, where $\boldsymbol{p}$ and $\boldsymbol{q}$ can represent either an arbitrary system component or the environment.

Ascendency: This is a key property of a network of flows that quantifies both the level of system activity and the degree of organization (constraint) with which material is being processed in autocatalytic systems such as ecosystems. The ascendency, $\boldsymbol{A}$, expressed in terms of trophic exchanges, $\boldsymbol{T_{ij}}$, from taxon $\boldsymbol{i}$ to taxon $\boldsymbol{j}$ is calculated as:

$$A = \sum_i \sum_j T_{ij} \log \left[\frac{T_{ij} T_{..}}{T_{.j} T_{i.}} \right]$$

where a dot as a subscript indicates summation over that index.

Development Capacity: This index is a surrogate for the complexity of the food web (Monaco & Ulanowicz, 1997). In other words, it is the diversity of the system flows scaled by the total system throughput. Quantitatively, it takes the form:

$$C = \sum_{i,j} T_{ij} \log \left[\frac{T_{ij}}{T_{..}} \right]$$

Average Mutual Information (AMI): Measures the average amount of constraint exerted upon an arbitrary quantum of currency as it is channelled from any one compartment to the next (Ulanowicz, 1997). It is the unscaled form of the ascendency and is written as:

$$AMI = \sum_{i,j} \frac{T_{ij}}{T_{..}} \log\left[\frac{T_{ij}T_{..}}{T_{i.}T_{.j}}\right]$$

Redundancy: This is the degree to which pathways parallel each other in a network. It can be calculated in an isolated system as the (non-negative) difference by which the system capacity exceeds the ascendency. In terms of flows it comes:

$$R = -\sum_{i,j=0}^{n} \frac{T_{ij}}{T_{..}} \log\left[\frac{T_{ij}^2}{T_{i.}T_{.j}}\right]$$

where n is the number of components in the system (for more details see Ulanowicz & Norden, 1990; Ulanowicz & Wulff, 1991).

Specific Overhead of the system (**Ø/TST**): It measures the total flexibility of the system on a per- unit- flow basis. The overhead of a system is the amount by which the capacity of a non-isolated system exceeds the ascendency. It consists mostly of redundancy, but in open systems it is also augmented by multiplicities in the external inputs and outputs. In terms of the flows it resembles the redundancy, only it also includes the transfers with the external world:

$$\Phi \big/ TST = -\sum_{i,j=0}^{n+2} \frac{T_{ij}}{T_{..}} \log\left[\frac{T_{ij}^2}{T_{i.}T_{.j}}\right]$$

where the index ($n+1$) signifies an import and ($n+2$) an export or dissipation.

b) Trophic analysis

Food webs that are qualitatively very different can be mapped into a standard straight-chain network topology. This standard form allows comparing corresponding trophic efficiencies between different estuaries (Baird *et al.* 1991).

The trophic efficiency between any two levels is defined as the amount a given level passes on to the next one, divided by how much it received from the previous level (Ulanowicz & Wulff, 1991). Connectance indices (overall connectance and intercompartmental connectance) are estimates of the effective number of links both into and out of each compartment of a weighted network.

c) Cycle analysis

The Finn Cycling Index (FCI) reveals the proportion of total system throughput that is devoted to the recycling of carbon (Finn, 1976). Thus, ***FCI=Tc/TST***, where ***Tc*** is the amount of system activity involved in cycling.

Patrício *et al.* (2004) applied ascendency to data on the Mondego estuarine intertidal communities showing that network analysis appeared to provide a systematic approach to apprehending what is happening at the whole--system level, which is obviously powerful from the theoretical point of view. Moreover, the study on the Mondego estuarine ecosystem provided an example of how the measures coming out of network analysis can lead to an improved understanding of eutrophication process itself. Nevertheless, there is a major inconvenient regarding its use, that is the extremely considerable time and labour needed to collect all the data necessary to perform network analysis, which limits it application.

2.4. Brief review of socio-economic indicators. The case of coastal environments

In general, human activities generate a series of damages and environmental stresses, which become evident in the alteration of natural processes that take place in different ecosystems. On the other hand, on top of environmental concerns, social, cultural and economical problems are overlapped, meaning that human activities are never isolated or disturb the environment through cause effect linear relations. Instead, they interact, meet and compete for the areas, summing effects up and producing a complex net of interrelations which make even more difficult to analyse the situations.

The United Nations (UN) developed a list of environmental indicators in collaboration with the Inter-governmental Working Group on the Advancement of Environment Statistics, and the fourth meeting of the Working Group (Stockholm, 6-10 February 1995) agreed on a list of environmental and related socio-economic indicators, which is provided in Table 10.

On the other hand, regarding coastal environments, issue specific global programs such as the *Millennium Ecosystem Assessment* and the *World Commission on Protected Areas Marine Program*, which follow an integrated approach or perspective with a focus on ecosystems and on marine protected areas, respectively, have developed different socio-economic indicators. These programs look at both environmental and socio-economic aspects and their interactions.

Table 10

Socieconomic activities and their impacts on the environment.

(source http://unstats.un.org/unsd/ENVIRONMENT/indicators.html)

	Socio-Economic Activitie and Events	**Impacts and Effects**
Economic Issues	Real GDP per capita growth rate Production and consumption patterns Investment share in GDP	EDP/EVA per capita Capital accumulation (environmentally adjusted)
Social/Demographic Issues	Population growth rate Population density Urban/rural migration rate Calorie supply per capita	% of urban population exposed to concentrations of SO_2, particulates, ozone, CO and Pb Infant mortality rate Incidence of environmentally related diseases
Air/Climate	Emissions of CO_2, SO_2 and NO_x Consumption of ozone depleting substances	Ambient concentrations of CO, SO_2, NO_x O_3 and TSP in urban areas Air quality index

Table 10 *(Continued)*

	Socio-Economic Activities and Events	Impacts and Effects
Land/Soil	Land use change Livestock per km^2 of arid and semi-arid lands Use of fertilizers Use of agricultural pesticides	Area affected by soil erosion Land affected by desertification Area affected by salinization and water logging
Fresh Water Resources	Industrial, agricultural and municipal discharges directly into freshwater bodies Annual withdrawals of ground and surface water Domestic consumption of water per capita Industrial, agricultural water use per GDP	Concentration of lead, cadmium, mercury and pesticides in fresh water bodies Concentration of fecal coliform in fresh water bodies Acidification of fresh water bodies BOD and COD in fresh water bodies Water quality index by fresh water bodies Deviation in stock from maximum sustainable yield of marine species Loading of N and P in coastal waters
Marine Water Resources	Industrial, agricultural and municipal discharges directly into marine water bodies Discharges of oil into coastal waters	Deviation in stock from maximum sustainable yield of marine species Loading of N and P in coastal waters
Biological Resources	Annual roundwood production Fuelwood consumption per capita Catches of marine species	Deforestation rate Threatened, extinct species
Mineral (incl. Energy) Resources	Annual energy consumption per capita Extraction of other mineral resources	Depletion of mineral resources (% of proven reserves) Lifetime of proven reserves
	Municipal waste disposal Generation of hazardous waste Imports and exports of hazardous wastes	Area of land contaminated by toxic waste
Human Settlements	Rate of growth of urban population % of population in urban areas Motor vehicles in use per 1000 habitants	Area and population in marginal settlements Shelter Index % of population with sanitary services
Natural Disasters	Frequency of natural disasters	Cost and number of injuries and fatalities related to natural disasters

UNESCO (2003) summarised these socio-economic indicators and those considered by national, state, local or site specific coastal management programs. In this summary we may find indicators focusing on the following approaches:

a) Coastal population (population density and population in coastal high hazard areas);

b) Quality of life in the coastal zone (unemployment levels, perceived quality of coastal landscape, availability of affordable housing, and population age structure);

c) Public information awareness (public awareness of coastal issues and public awareness of sustainable development);

d) Public access (legal availability, and access points);

e) Service needs and provision (education, health, welfare, housing, water and sanitation, electricity, wastewater and stormwater, roads, railways, airports and harbours, telecommunication and postal services);

f) Tourism and recreation (value of tourism and employment in the tourism sector, importance of tourism to the economy, tourist arrivals, equitable distribution of tourism benefit);

g) Fisheries (annual catch of major target species, percentage of household income derived from fishing);

h) Coastal community development (environment and land use, economic diversity and positive and negative economic growth, engagement between government and the public, public investment and infrastructures);

i) Development funding (level of finance from multilateral institutions and other institutional funding institutions);

j) Coastal dependent uses (description of the authority to enact laws and ordinances to protect public health, safety and welfare, and economic health measured by the different types and trends in economic development);

k) Community participation (number of people involved in coastal management activities and extent of participation, level of awareness of coastal issues, business participation in coastal management activities, participation in volunteer activities that protect and enhance coastal resources);

l) Coastal hazards (population in coastal high hazards areas, emergency evacuation, shelter demand, and capacity, level of awareness of coastal hazards, number of reported vessel incidents and land acquired for hazard mitigation);

m) Waterfront revitalisation (number of volunteers contributing time to activities associated with waterfront revitalisation, public and private investment in waterfront communities, number of community goals achieved).

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CHAPTER 3

DECISION TREE FOR SELECTING ECOLOGICAL INDICATORS BASED ON BENTHIC INVERTEBRATE FAUNA DATA SETS

At least in theory, all ecological indicators accounting for the species composition and abundance of biological communities might be useful in characterising the environmental situation of an ecosystem. However, since many indicators were in practice developed to approach the characteristics of a specific ecosystem, they often lack generality. On the other hand, many 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. To decide on the set of ecological indicators to use in a particular case is therefore a sensible process.

In the process of selecting an ecological indicator, the type of pollution, type of community, data requirements and data availability must be accounted for. Moreover, the complementary use of different indicators or methods based on different ecological principles is highly recommended in determining the environmental quality status of an ecosystem (Dauer *et al.*, 1993).

There are numerous kinds of pollution in the marine environment. One classification could be made according to the nature of the pollutant, having then pollution caused by toxic substances, toxic thermal, radioactive, organic, and microbiological, as well as those other types which imply changes in the dynamics of the marine environment functioning without having a specific characterisation: suspended materials, fresh water input, etc. Among these types, organic and toxic pollution and physical disturbances are the most frequent perturbations in coastal areas.

Pollution caused by toxic substances may affect negatively the organisms' physiology, producing even their death. Such substances are usually characterised as to be highly persistent and cause bioaccumulation in organisms via trophic networks. This provokes serious problems even at low concentrations, interfering the enzymatic function and inducing the production of mixed-function oxidases (Kurelec *et al.,* 1984; Spies *et al.,* 1984; Hansen & Addison, 1990; Lafaurie *et al.,* 1993) or metalotioneines (Harrison *et al.,* 1988; Carpene, 1993; Roesijadi, 1994; Stewart, 1994; Ringwood *et al.,* 1995). Among the toxic substances most frequently found in the marine environment are organochlorates, characterised by high persistence to chemical and bacterial degradation, biphenyl-chlorates, dioxins, etc. Their presence in the sea is commonly associated to agricultural and industrial activities.

Heavy metals constitute another particular case of toxic pollution. They proceed from mining drainage, industrial waste discharges, as well as mud from sewage treatment plants, and direct dumping from metal application and transformation industries. Heavy metals can be adsorbed by mineral and organic particles that tend to drag them to the sea bottom where they stay, retained in the sediment, or they become assimilated by living organisms that incorporate them to the trophic chains, causing bioaccumulation.

Organic pollution can be claimed as the most generalised, without any hesitation, and can be defined as the presence in the marine environment of organic substances that in principle are capable of being biodegraded and assimilated by the system. The volume of organic matter dumped in our coasts exceeds that of any other pollutant, as any urban settlement, whatever the small size it could be, is capable of producing large quantities of organic waste. Such type of pollution is usually associated to urban drainage network dumping, although agricultural activity, through fertilizers usage, and other different kinds of industries, namely agroalimentary, paper, and fish farming contribute in a very important way to the organic enrichment of the coastal environment.

On the other hand, the main physical stressors consist of mechanical disturbance (*e.g.* fishing), removal of substratum *(e.g.* aggregated or dredging), changes in grain size, changes in temperature, suspended sediment, water flow rates, and sediment deposition (smothering).

Assessing the effect of any disturbance on the biological component of the ecosystem or community is very often dependent on the type of data available. Moreover, at the same time, applying different ecological indicators depends on a series of requisites.

Taking into account the indicators more frequently applied in marine studies, we provided a friendly decision tree (Figure 2) to be used in selecting the most suitable indices or ecological indicators for each case, except for merely graphical methods due to its high subjectivity. We bore in mind the most frequent types of disturbance occurring in coastal areas, namely organic enrichment, physical disturbance (mechanical disturbance and removal of substratum) or toxic pollution, and also the required level of organisms taxonomic identification, and the type of substrate.

We must highlight that a decision tree of this type is never concluded. For the sake of this work, we have included the indicators most used in the literature until the end of 2004. However, new indices can be considered in the structure of the decision tree following the selection criteria proposed.

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Decision tree for selecting the most adequate indices or ecological indicators as a function of the type of disturbance and benthic invertebrate fauna data availability:

1. The type of perturbation whose impact we intend to assess consists of toxic pollution. . 2
The type of perturbation whose impact we intend to assess consists of organic enrichment or physical disturbance. 5

2. The effect of pollution is to be evaluated at the level of communities' structure. 5
The effect of pollution is to be evaluated regarding contaminant's toxic potential. 3
The toxic reduction in organisms is determined with the only intention of developing comparative measurements. .... **ERI**

3. Contamination is caused by heavy metals. ....**Metallothionein Induction Method**
ALA D Inhibition (Pb) Method
Lysosomal Stability Method
Lysosomal Neutral Red Retention Method
Contamination is caused by others than heavy metals. 4

4. Contamination is caused by xenobiotics. ....**Lysosomal Stability Method**
Lysosomal Neutral Red Retention Method
Contamination is caused by organotins ....**Shell Thinkening Method**
Imposex Method
Intersex Method

5. Biological data available refers to a benthic meiofauna community:...................................
Nematodes/Copepods Index
Meiobenthic Pollution Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness
Biological data available refers to benthic macrofauna community 6

6. Our concern is the benthic macrofaunal community and the type of data available includes *no* information on physico-chemical parameters. 7
Our concern is the benthic macrofaunal community and the type of data available includes information on physico-chemical parameters. 10

7. The type of data available includes information on biomass of organisms sampled. 8
The type of data available *does not* include information on biomass of organisms sampled.... 9

8. Organisms are identified up to the species level ....**AMBI**
BENTIX
Indicator Species Index
Pollution Index (Bellan)
Pollution Index (Bellan-Santini)
Infaunal Trophic Index
Polychaetes/Amphipods Ratio (recommended in case of oil pollution)
Feeding Structure Index
Mollusc Mortality Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness
W-Statistic (not recommended in case of physical disturbance and/or toxic pollution)
Exergy
Specific Exergy
Organisms are identified up to the genus or family level........**Polychaetes/Amphipods Ratio**
Mollusc Mortality Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness
W-Statistic (not recommended in case of physical disturbance and/or toxic pollution)
Exergy
Specific Exergy

9. The type of data available includes information on numerical density of organisms sampled and organisms are identified up to the species level..**AMBI**
BENTIX
Indicator Species Index
Pollution Index (Bellan)

Pollution Index (Bellan-Santini)
Infaunal Trophic Index
Polychaetes/Amphipods Ratio (recommended in case of oil pollution)
Feeding Structure Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness

The type of data available includes information on numerical density of organisms sampled and organisms are identified up to the genus or family level .... **Polychaetes/Amphipods Ratio**
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness

10. The type of data available includes information on biomass of organisms sampled. ... 11
The type of data available does not include information on biomass of organisms sampled. 12

11. Organisms are identified up to the species level .... **AMBI**
BENTIX
Indicator Species Index
Pollution Index (Bellan)
Pollution Index (Bellan-Santini)
Infaunal Trophic Index
Polychaetes/Amphipods Ratio (recommended in case of oil pollution)
Feeding Structure Index
Mollusc Mortality Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness
W-Statistic (not recommended in case of physical disturbance and/or toxic pollution)
Exergy
Specific Exergy
Pollution Coefficient
B-IBI

Benthic Index of Environmental Condition

Organisms are identified up to the genus or family level........ **Polychaetes/Amphipods Ratio**

Mollusc Mortality Index

Shannon-Wiener Index

Pielou Index

Margalef Index

Berger-Parker Index

Simpson Index

Taxonomic Diversity

Taxonomic Distinctness

W-Statistic (not recommended in case of physical disturbance and/or toxic pollution)

Exergy

Specific Exergy

Pollution Coefficient

Benthic Index of Environmental Condition

12. The type of data available includes information on numerical density of organisms sampled and organisms are identified up to the species level..**AMBI**

BENTIX

Indicator Species Index

Pollution Index (Bellan)

Pollution Index (Bellan-Santini)

Infaunal Trophic Index

Polychaetes/Amphipods Ratio (recommended in case of oil pollution)

Feeding Structure Index

Shannon-Wiener Index

Pielou Index

Margalef Index

Berger-Parker Index

Simpson Index

Taxonomic Diversity

Taxonomic Distinctness

Pollution Coefficient

Benthic Index of Environmental Condition

The type of data available includes information on numerical density of organisms sampled and organisms are identified up to the genus or family level .... **Polychaetes/Amphipods Ratio**

Shannon-Wiener Index

Pielou Index

Margalef Index

Berger-Parker Index

Simpson Index

Taxonomic Diversity

Taxonomic Distinctness

Pollution Coefficient

Benthic Index of Environmental Condition

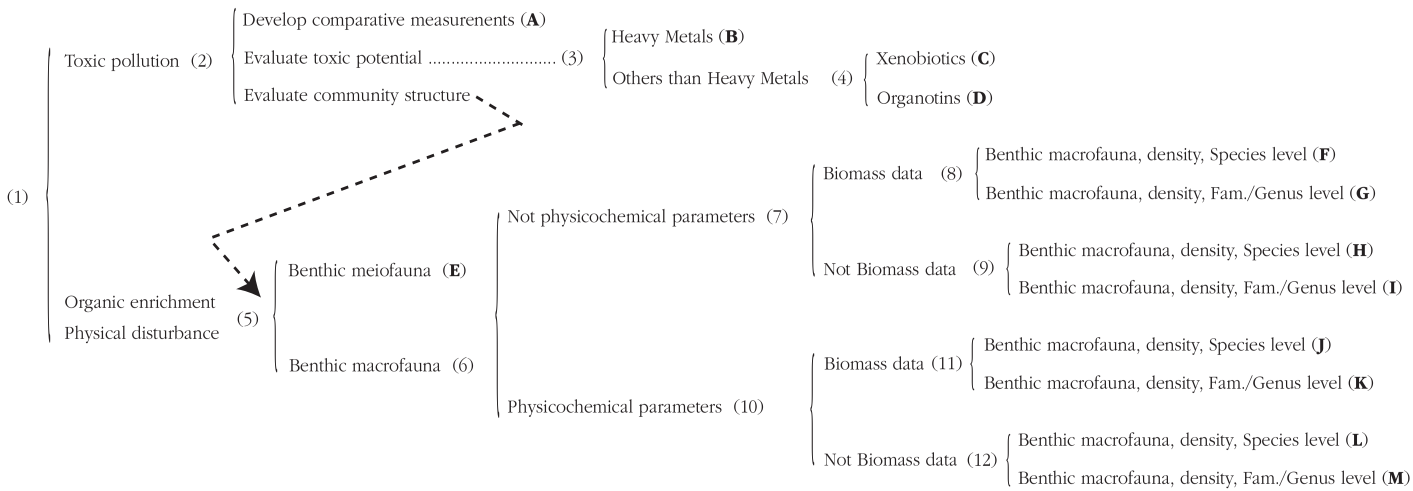

Figure 2. Decision tree for selecting ecological indicators based on benthic invertebrate fauna data sets. ERI: Ecological Reference Index; N/C: Nematodes/Copepods Index; MPI: Meiobenthic Pollution Index; S-W: Shannon-Wiener Index; P: Pielou Index; M: Margalef Index; B-P: Berger-Parker Index; Simp: Simpson Index; TDiv: Taxonomic Diversity; TDist: Taxonomic Distinctness; ISI: Indicator Species Index; PI: Pollution Index (Bellan); PI (B-S): Pollution Index (Bellan-Santini); ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods Ratio; FSI: Feeding Structure Index; MMI: Mollusc Mortality Index; Ex: Exergy; Sp-Ex: Specific-Exergy; Pol Coef: Pollution Coefficient; BIEC: Benthic Index of Environmental Condition.

(**A**): ERI

(**B**): Metallothionein Induction Method, ALA D Inhibition (Pb) Method, Lysosomal Stability Method, Lysosomal Neutral Red Retention Method

(**C**): Lysosomal Stability Method, Lysosomal Neutral Red Retention Method

(**D**): Shell Thickening Method, Imposex Method, Intersex Method

(**E**): N/C, Meiobenthic PI, S-W, P, M, B-P, Simp, TDiv, TDist

(**F**): AMBI, BENTIX, ISI, PI, PI (B-S), ITI, P/A, FSI, MMI, S-W, P, M, B-P, Simp, TDiv, TDist, W-Statistic, Ex, Sp-Ex

(**G**): P/A, MMI, S-W, P, M, B-P, Simp, TDiv, TDist, W-Statistic, Ex, Sp-Ex

(**H**): AMBI, BENTIX, ISI, PI, PI (B-S), ITI, P/A, FSI, S-W, P, M, B-P, Simp, TDiv, TDist

(**I**): P/A, S-W, P, M, B-P, Simp, TDiv, TDist

(**J**): AMBI, BENTIX, ISI, PI, PI (B-S), ITI, P/A, FSI, MMI, S-W, P, M, B-P, Simp, TDiv, TDist, W-Statistic, Ex, Sp-Ex, Pol Coef, B-IBI, BIEC

(**K**): P/A, MMI, S-W, P, M, B-P, Simp, TDiv, TDist, W-Statistic, Ex, Sp-Ex, Pol Coef, BIEC

(**L**): AMBI, BENTIX, ISI, PI, PI (B-S), ITI, P/A, FSI, S-W, P, M, B-P, Simp, TDiv, TDist, Pol Coef, BIEC

(**M**): P/A, S-W, P, M, B-P, Simp, TDiv, TDist, Pol Coef, BIEC

CHAPTER 4

DECISION TREE FOR SELECTING ADEQUATE INDICES OR ECOLOGICAL INDICATORS: EXAMPLES OF APPLICATIONS

To illustrate the application of this decision tree for selecting the most suitable indices or ecological indicators as a function of the type of disturbance and data availability we have used data from five study areas, two of them located in the Western coast of Portugal and three areas located at the South-Eastern coast of Spain.

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 in the community were available. The fact of having at our disposal such a set of heterogenous raw data represented an excellent opportunity to show how the exercise of applying the proposed key should be carried out.

4.1. Description of the study areas and type of data available

4.1.1. The Mondego estuary

The Mondego estuary is located on the western coast of Portugal and it consists of two arms, north and south, which become separated by an island at about 7 km from the sea, getting together again near the mouth (Figure 3). Different hydrographical characteristics are found in the two arms. The north arm is deeper than the south arm, which is at present totally silted up in the upstream areas, which causes the freshwater of the river to flow essentially through the north arm. The water circulation in the south arm is dependent on tidal activity and on small freshwater input from a tributary, the Pranto river, which is controlled by a sluice (Marques *et al.*, 2003). Harbour facilities and dredging activities, on the north arm, cause physical disturbance of the bottoms, while freshwater discharges from agricultural areas in the river valley result in an excessive nutrient release into the south arm (Marques *et al.*, 1993). Human pressure coupled with specific physical characteristics (water residence time, hydrodynamics and depth) and climate conditions (precipitation) have contributed to an increase of environmental stress in the Mondego estuary (Dolbeth *et al.*, 2003). Nevertheless, recently, the system appears to be gradually recovering from the effects of eutrophication processes that harassed it over the past two decades (Pardal *et al.*, 2004).

Figure 3. The Mondego estuary, Portugal.

Two different data sets were selected to estimate different ecological indicators:

a) The first one was provided by a study on the subtidal soft bottom communities, which allowed characterising the whole system with regard to species composition and abundance, taking into account its spatial distribution in relation to the physicochemical factors of water and sediments. The benthic macrofauna was sampled twice during spring, in 1990, 1992, 1998 and 2000, at 14 stations covering the whole terminal part of the system (Figure 4A);

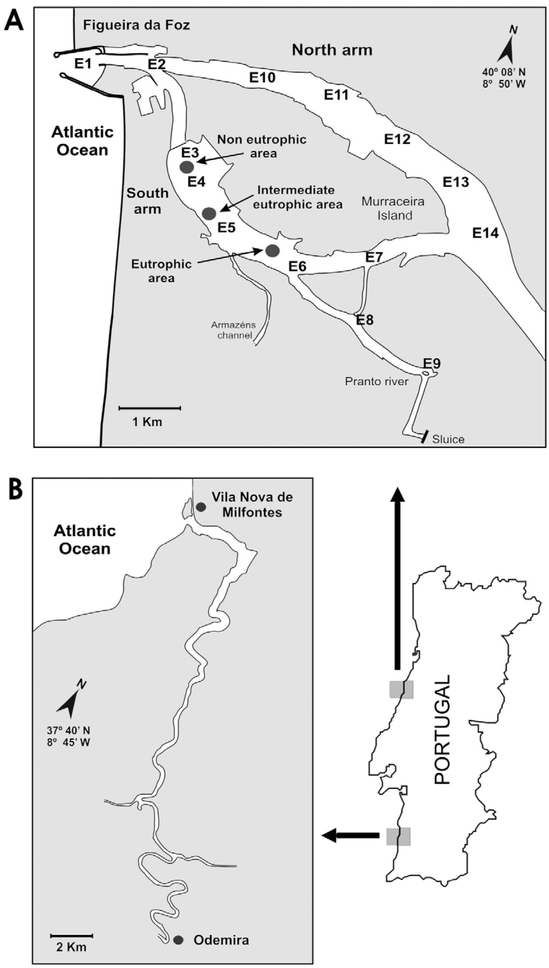

Figure 4. Portuguese case studies location.
A: Mondego estuary, B: Mira estuary and sampling stations.

b) The second one proceeded from a study on the intertidal benthic communities carried out from February 1993 to June 1994 in the south-arm of the estuary (Figure 4A). Samples of macrophytes, macroalgae, and associated macrofauna, as well as samples of water and sediments, were taken fortnightly at different sites, during low tide, along a spatial gradient of eutrophication symptoms, from a non eutrophic zone, where a macrophyte community (*Zostera noltii*) is present, up to an eutrophic one, in the inner areas of the estuary, from where the macrophytes disappeared while *Ulva* sp. (green macroalgae) blooms have been observed during the last decade. In this area, as a pattern, *Ulva* sp. biomass normally increases from early winter (February/March) up to July, when an algal crash usually occurs. A second but much less important algal biomass peak may sometimes be observed in September, followed by a decrease up to winter (Marques *et al.*, 1997).

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

4.1.2. The Mira estuary

The Mira estuary is 32 Km long, extending from Vila Nova de Milfontes, located close to the mouth, up to Odemira, at its upper limit (Figure 5). Both are small towns, constituting nevertheless the most important urbain centres in the whole basin region. In general, the estuary is narrow and entrenched, with approximately 150 m in width at its lower part and only 30 m in the upper reaches, with a mean depth of about 6 m (Costa *et al.*, 1994).

The Mira estuary and the surrounding area are included in the Natural Park of Sudoeste Alentejano and Costa Vicentina. The landscape is characterised by irrigated fields, well-preserved eucalyptus and cork-oak woods and undergrowth (Raposo, 1996). The prevailing conditions allow, to a certain extent, to consider the Mira estuary as representing a pristine system.

The selected data are included in the data base developed by the TICOR project (Bettencourt *et al.*, 2004), and come from a study worked out by Andrade (1986) in which 99 sampling stations were utilised, covering the whole system (Figure 4B). Only data on abundances of benthic organisms are available.

Figure 5. The Mira estuary, Portugal.

4.1.3. The Mar Menor lagoon

The Mar Menor is a coastal lagoon with an area of 135 Km^2. The lagoon is connected to the Mediterranean at some points by channels through which the water exchange takes place with the open sea (Figure 6).

The Mar Menor biological communities are adapted to more extreme temperatures and salinities than those found in the open sea. This coastal lagoon presents an environmental heterogeneity with different types of organic pollution. Some areas are affected by: 1) urban direct dumping with the development of nitrophyle communities dominated by Ulvae species; 2) dumping or zones under the influence of harbours; 3) zones with high levels of organic matter in the sediment coming from the primary production and the biological cycle of the macrophyte meadows (*Caulerpa prolifera*). This macrophyte was introduced in the lagoon as the result of the dredging in one of the channels at the beginning of the 70s, growing rapidly around the whole lagoon, phenomenon which has been accelerated in the last years. Such *Caulerpa prolifera* growth has led to an increment of the organic matter in the sediment. Such increment, although it has a natural origin, had important consequences in the communities, with a general fauna impoverishment. In that sense, the named increment can be considered an authentic pollution as it is understood by the GESAMP; 4) zones with low input of organic matter in the soft substrates (>1%) and on rocky bottoms.

Figure 6. The Mar Menor coastal lagoon, Spain.

To estimate different ecological indicators we used data from Pérez-Ruzafa (1989), as they have the advantage of constituting a complete characterisation of the lagoon's benthic populations, containing all the information needed for a study as the present one. Eleven sampling stations were located on rocky and soft substrates along the lagoon at sites representative of the different biocenosis and main polluted areas (Figure 7A). In some of the stations samples were taken seasonally (A: July, B: November, C: February, D: May) in order to evaluate the independence of different ecological indices with regard to seasonal variations.

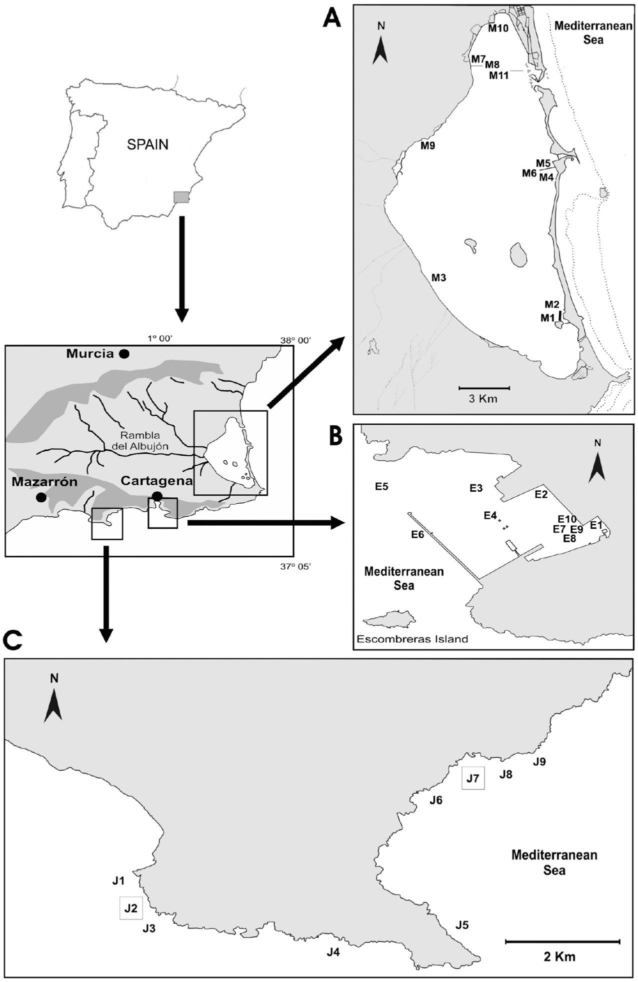

Figure 7. Spanish case studies location. A: Mar Menor coastal lagoon, B: Escombreras basin, C: Cape Tiñoso and sampling stations (squares around stations represent cages).

Likewise the Mondego estuary case study, organisms were identified to the species level and their biomass was determined (gm^{-2}AFDW). The environmental factors taken into account were salinity, temperature, pH, and dissolved oxygen, as well as sediment particles size, organic matter and heavy metal contents.

4.1.4. Escombreras basin

The dock of Escombreras is located in the inlet of the same name, marked it out by Del Gate point in the North extreme and by Aguilones point and the Escombreras Island in the South. This natural bay is closed by the Bastarreche dike-port and a series of docks and wharves subject to great use can be found in the inner areas (Figure 8).

Figure 8. Escombreras basin. A. Aereal photograph; B. Industrial complex situated in the basin.

The Escombreras inlet, due to its own geographical characteristics and the proximity to the port city of Cartagena, has been developing as an important centre of maritime transport which serves numerous factories situated in the Escombreras Valley. As a consequence, the dumping and waste produced by such activity became a conditioning factor, which has been modelling the characteristics of the bay and the biological communities present in there.

Most of the dumping of industrial and domestic wastes in the area have been carried out into the marine environment, right in the bay or in very close places around, and it can be assured that the marine communities as well as the physico-chemical characteristics of both the waters and the marine sediments have been altered and modified in the last years as a consequence of such dumping.

Data from the Escombreras dock were collected in the scope of an integrated study on pollution characterisation carried out in the area in 1994 (Pérez-Ruzafa *et al.*, 1994).

Data were collected at 10 sampling stations (Figure 7B), in July 1994, describing the subtidal soft-bottom communities along the whole system with regard to species composition and abundance. A large number of pollutants proceeding from waste and industrial dumping could be identified in the area, which have led to an alteration of the physical and chemical characteristics of the waters and marine sediments.

4.1.5. Cape Tiñoso

Cape Tiñoso is located between La Azohía point and Aguilones Point (Figure 9). The biocenosis in the area is characterised by a high specific diversity and a great structural maturity, with a well developed and preserved *Posidonia oceanica* meadow (C.A.R.M., 1998).

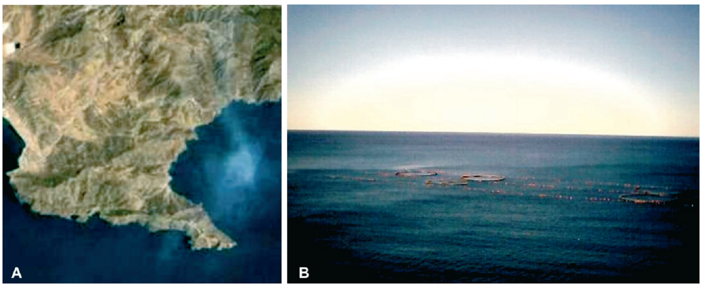

Figure 9. Cape Tiñoso. A. Aereal photograph;
B. Floating cages assigned to red tuna fattening.

The high ecological value of the area is related with a low tourism pressure as compared to other zones of the Murcian littoral. Fishing activities are the only ones clearly developing from 1996, when the first floating cages assigned to red tuna fattening were installed, and reached nowdays a considerable added value. The environmental effects of such type of activity consist of a) increment in water turbidity, b) increment of water dissolved nutrients, and c) direct organic enrichment of sediments due to faeces of the cultivated organisms and excess in food supply, with the corresponding ecological related consequences.

Data from Tiñoso Cape were provided by a surveillance and monitoring programme of the environmental impact of fish farming activities (Pérez-Ruzafa *et al.*, 1997). Nine sampling station were establish in the area assumed to be affected by the cages' influence, and two control stations in the eastern most extreme of Cape Tiñoso (Figure 7C).

One of the cages fields is located between 37º 33' 5" N, 37º 33' 25" N and 1º 10' 15" E, near by La Azohía point, about 20 to 40 metres depth. A second field from the factory is located between 37º 33' 50" N, 37º 34' 10" N and 1º 6' 5" E y 1º 6' 30" E, near Aguilar and Bolete beaches, 25 to 40 metres depth. Sampling stations in the area under the influence of the cages were positioned in such a way that, with a single exception, all were in the course of the two dominant currents, the first with 0.38 knots of average speed, in the area of the first field of cages, and another with 0.33 knots of average speed, in the second one. Moreover, two sampling stations were located exactly under the cages' fields (Figures 7C and 9).

At all stations samples were taken in August and November 1996, February, June and November 1997. At each time, the organic matter content in the sediments and the granulometry were determined, as well as the concentration of nutrients in the water column.

4.2. Indicators selection as a function of the available data

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 11 provides a summary of the main disturbance factors and the type of data available regarding each one of our five case studies. In case we do not know the type of disturbance (like in the case of the Mira estuary) it is advisable to choose option 1 (organic enrichment) as it includes more non pollution specific ecological indicators.

The next step is choosing 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 be taken into account that among indicators based on the same principles, we should choose the ones which best include the characteristics that define a good ecological indicator.

Table 11

Main disturbance factors and type of available data regarding the five case studies.

Study Area	Principal Disturbance Factor	Type of Data
Mondego estuary	Organic enrichment	Abundance of benthic organisms
		Biomass of benthic organism (in the case of subtidal communities, 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)

For instance, among the indices based on indicator species, we may have the pollution indices of Bellan, Bellan-Santini, BENTIX, Indicator Species Index and 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 suitable. In fact, BENTIX is too much specific for Mediterranean coastal waters, namely for areas near Greece, and the Indicator Species Index is particular 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 decision tree, 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.

We will show afterwards how, with the help of the proposed decision tree, we can select which ecological indicators (indicated in bold) appear more recommendable for application in the five case studies considered, as a function of the type of disturbance and available data and, also, *a priori* knowledge regarding their characteristics.

***Example 1**: The Mondego estuary*

1. The type of perturbation whose impact we intend to assess consists of organic enrichment or physical disturbance. 5

5. Biological data available refers to benthic macrofauna community 6

6. Our concern is the benthic macrofaunal community and the type of data available includes information on physico-chemical parameters. 10

10. The type of data available includes information on biomass of organisms sampled. ... 11

11. Organisms are identified up to the species level .... **AMBI**
BENTIX
Indicator Species Index
Pollution Index (Bellan)
Pollution Index (Bellan-Santini)
Infaunal Trophic Index
Polychaetes/Amphipods Ratio
Feeding Structure Index
Mollusc Mortality Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness Measures
W-Statistic
Exergy
Specific Exergy
Pollution Coefficient
B-IBI
Benthic Index of Environmental Condition

Example 2: The Mira estuary

1. The type of perturbation whose impact we intend to assess consists of organic enrichment or physical disturbance. 5

5. Biological data available refers to benthic macrofauna community 6

6. Our concern is the benthic macrofaunal community and the type of data available includes no information on physico-chemical parameters. 7

7. The type of data available **does not** include information on biomass of organisms sampled. ..9

9. The type of data available includes information on numerical density of organisms sampled and organisms are identified up to the species level... **AMBI**
BENTIX
Indicator Species Index
Pollution Index (Bellan)
Pollution Index (Bellan-Santini)
Infaunal Trophic Index
Polychaetes/Amphipods Ratio

Feeding Structure Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness Measures

Example 3: The Mar Menor lagoon

1. The type of perturbation whose impact we intend to assess consists of organic enrichment or physical disturbance. 5

5. Biological data available refers to benthic macrofauna community 6

6. Our concern is the benthic macrofaunal community and the type of data available includes information on physico-chemical parameters. 10

10. The type of data available includes information on biomass of organisms sampled. ... 11

11. Organisms are identified up to the species level ....**AMBI**
BENTIX
Indicator Species Index
Pollution Index (Bellan)
Pollution Index (Bellan-Santini)
Infaunal Trophic Index
Polychaetes/Amphipods Ratio
Feeding Structure Index
Mollusc Mortality Index
Shannon-Wiener Index
Pielou Index
Margalef Index
Berger-Parker Index
Simpson Index
Taxonomic Diversity
Taxonomic Distinctness Measures
W-Statistic
Exergy
Specific Exergy
Pollution Coefficient
B-IBI
Benthic Index of Environmental Condition

***Example 4**: The Escombreras basin*

1. The type of perturbation whose impact we intend to assess consists of toxic pollution. . 2

2. The effect of pollution is to be evaluated at the level of communities' structure. 5

5. Biological data available refers to benthic macrofauna community 6

6. Our concern is the benthic macrofaunal community and the type of data available includes information on physico-chemical parameters. 10

10. The type of data available includes information on biomass of organisms sampled. ... 11

11. Organisms are identified up to the species level .... **AMBI**

BENTIX

Indicator Species Index

Pollution Index (Bellan)

Pollution Index (Bellan-Santini)

Infaunal Trophic Index

Polychaetes/Amphipods Ratio

Feeding Structure Index

Mollusc Mortality Index

Shannon-Wiener Index

Pielou Index

Margalef Index

Berger-Parker Index

Simpson Index

Taxonomic Diversity

Taxonomic Distinctness

W-Statistic

Exergy

Specific Exergy

Pollution Coefficient

B-IBI

Benthic Index of Environmental Condition

***Example 5**: The Cape Tiñoso*

1. The type of perturbation whose impact we intend to assess consists of organic enrichment or physical disturbance. 5

5. Biological data available refers to benthic macrofauna community 6

6. Our concern is the benthic macrofaunal community and the type of data available includes information on physico-chemical parameters. 10

10. The type of data available includes information on biomass of organisms sampled. ... 11

11. Organisms are identified up to the species level ....**AMBI**

BENTIX

Indicator Species Index

Pollution Index (Bellan)

Pollution Index (Bellan-Santini)

Infaunal Trophic Index

Polychaetes/Amphipods Ratio

Feeding Structure Index

Mollusc Mortality Index

Shannon-Wiener Index

Pielou Index

Margalef Index

Berger-Parker Index

Simpson Index

Taxonomic Diversity

Taxonomic Distinctness Measures

W-Statistic

Exergy

Specific Exergy

Pollution Coefficient

B-IBI

Benthic Index of Environmental Condition

4.3. Results of the application

4.3.1. The Mondego estuary

Firstly, the analysis was focused on the subtidal communities from both arms of the estuary (first data set). Table 12 summarises the indices values obtained.

Of all the indicators utilised, the only ones able to discriminate between different areas in the Mondego estuary are the Margalef, Total Taxonomic

Table 12

Indices values in Mondego estuary (subtidal) stations in 1990, 1992, 1998 and 2000. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ⁺: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	E1				E2				E3				E4				E5				E6				E7			
	1990	1992	1988	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000
AMBI	–	–	1.2	1.53	2.62	1.58	1.87	3.22	2.11	1.90	3.00	2.45	2.86	2.50	2.76	2.39	3.08	3.18	3.04	3.04	3.04	3.07	2.84	2.99	2.00	3.07	3.00	3.05
FSI	–	–	0.75	1.67	0.33	0.40	0.25	0.25	0.33	0.25	0	0.40	0.13	0.20	0.20	0.17	0.29	0.11	0.13	0.11	0.13	0.29	0.17	0.13	0.22	0.33	0.17	0.11
ITI	–	–	81.4	95.96	79.70	59.05	45.85	51.52	50.08	70.82	33.34	54.55	62.91	74.08	60.25	56.67	66.67	64.65	58.57	67.88	65.49	61.68	63.77	68.99	66.67	66.67	57.31	69.79
Shannon-Wiener	–	–	2.85	0.87	1.56	1.74	2.45	3.45	2.56	2.94	0	2.55	3.11	2.42	1.43	2.92	1.22	2.74	2.03	2.51	0.72	1.88	1.91	1.46	1.61	1.44	1.66	2.39
Pielou	–	–	0.73	0.26	0.78	0.62	0.95	0.73	0.85	0.88		0.91	0.75	0.94	0.55	0.97	0.29	0.82	0.64	0.73	0.22	0.59	0.60	0.46	0.45	0.72	0.59	0.72
Margalef	–	–	2.32	1.30	0.91	1.26	1.35	4.01	1.47	2.01	0	1.74	3.15	1.47	0.94	1.99	2.11	1.91	1.07	1.35	1.18	1.32	1.25	1.03	1.57	0.71	0.81	1.43
Berger-Parker	–	–	0.43	0.87	0.62	0.66	0.30	0.25	0.34	0.23	1.00	0.36	0.41	0.33	0.73	0.20	0.81	0.33	0.61	0.37	0.90	0.54	0.43	0.69	0.66	0.65	0.61	0.42
Simpson	–	–	0.23	0.76	0.41	0.45	0.18	0.15	0.20	0.15	1.00	0.18	0.20	0.18	0.55	0.11	0.67	0.19	0.39	0.22	0.81	0.37	0.35	0.52	0.48	0.46	0.42	0.25
W-Statistic	–	–	0.27	- 0.19	–	–	0.40	0.20	–	–	-1	0.45	–	–	-0.15	0.50	–	–	-0.06	0.24	–	–	0.22	0.06	–	–	-0.04	0.11
Δ	–	–	71.94	22.93	55.99	47.64	73.64	73.58	72.48	71.73	0	75.95	72.02	76.61	42.88	82.11	28.19	76.88	49.52	67.82	16.03	61.11	51.87	46.73	42.56	51.6	56.22	69.71
Δ*	–	–	93.63	97.17	95.38	87.15	95.14	86.20	92.25	84.09	0	92.71	91.05	93.75	95.06	92.64	84.71	94.71	81.73	87.43	86.75	96.39	81.17	96.38	81.94	95.91	97.62	93.18
Δ⁺	–	–	92.38	85.56	97.22	83.33	88.33	90.51	90.48	87.04	0	91.27	90.69	91.11	94.44	92.26	87.15	91.48	88.43	88.89	92.59	86.57	89.88	86.57	93.64	94.44	92.86	90.74
TTD	–	–	1385.71	855.56	388.89	583.33	441.67	2353.33	633.33	870.37	0	638.89	1541.67	546.67	566.67	738.10	1568.63	914.81	795.83	888.89	833.33	779.17	719.05	779.17	1030.00	377.78	650.00	907.41
STTD	–	–	206.50	390.12	38.58	476.19	336.11	295.46	279.67	578.88	0	254.47	297.24	217.28	135.80	207.98	343.13	205.21	305.86	308.64	207.48	475.61	284.51	444.74	217.08	154.32	173.85	266.12
Exergy	–	–	214.08	3528.27	–	–	31.59	3424.53	–	–	5.76	15.04	_	–	6.53	31.09	–	–	33.29	427.15	–	–	15.31	307.04	–	–	310.90	85.18
Specific-Exerxy	–	–	99.75	276.30	–	–	218.84	217.43	–	–	450.00	65.44	–	–	159.35	348.10	–	–	165.58	215.13	–	–	10.98	200.52	–	–	119.26	82.85
B-IBI	–	–	3.67	3.67	–	–	2.67	3.00	–	–	2.33	3.00	–	–	1.67	2.67	–	–	2.33	3.00	–	–	2.00	3.00	–	–	2.33	2.67

Table 12 *(Continued)*

Indices values in Mondego estuary (subtidal) stations in 1990, 1992, 1998 and 2000. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ⁺: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	E8				E9				E10				E11				E12				E13				E14			
	1990	1992	1988	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000	1990	1992	1998	2000
AMBI	3.25	3.15	3.08	3.07	3.25	2.89	3.00	3.00	2.49	2.81	3.15	3.81	2.39	3.00	2.14	2.35	0.29	–	2.25	2.5	2.61	1.87	2.24	1.20	2.87	2.96	1.65	3.03
FSI	0.71	0.14	0.17	0.17	0.17	0	0	0.33	0.50	0	0	0.43	0	0.17	0	0.33	0	–	0	0	0.1	0	0		0	0.50	0	–
ITI	63.02	67.25	60.68	66.42	60.07	64.98	62.5	68.63	66.42	63.75	33.34	61.43	33.34	56.25	37.69	52.39	57.25	–	47.51	33.34	47.15	33.34	46.38	53.34	59.56	92.89	36.37	58.12
Shannon-Wiener	1.93	2.35	1.47	1.68	2.31	0.35	0.83	1.38	1.50	0.96	1.36	2.40	1.85	2.45	2.96	1.84	1.56	–	2.14	0.65	2.95	1.75	2.61	1.37	1.20	0.55	0.87	2.04
Pielou	0.61	0.78	0.46	0.53	0.73	0.22	0.36	0.59	0.95	0.42	0.68	0.72	0.92	0.82	0.89	0.92	0.60	–	0.76	0.65	0.82	0.88	0.87	0.86	0.52	0.35	0.55	0.73
Margalef	1.25	1.18	0.98	1.15	1.37	0.38	0.72	0.8	0.77	0.72	0.89	1.53	0.95	1.38	1.99	0.9	0.99	–	1.26	0.27	1.95	0.73	1.55	0.67	0.79	0.36	0.60	1.23
Berger-Parker	0.61	0.34	0.70	0.46	0.46	0.95	0.86	0.69	0.50	0.83	0.70	0.40	0.43	0.38	0.30	0.43	0.65	–	0.37	0.83	0.25	0.44	0.39	0.60	0.77	0.89	0.82	0.54
Simpson	0.4	0.24	0.52	0.38	0.27	0.9	0.74	0.51	0.32	0.69	0.50	0.25	0.27	0.23	0.15	0.28	0.47	–	0.26	0.72	0.16	0.32	0.21	0.41	0.60	0.81	0.67	0.34
W-Statistic	–	–	-0.2	-0.09	–	–	-0.18	0.24	–	–	0.21	0.23	–	–	0.59	0.39	–	–	0.3	-0.5	–	–	-0.05	0.20	–	–	0.18	0.19
Δ	47.72	72.55	40.85	47.63	62.18	10.09	24.59	42.66	63.13	25.28	47.65	44.5	59.72	69.56	74.27	67.70	39.49	–	69.02	28.48	78.67	65.28	73.57	58.92	35.36	19.30	32.58	57.11
Δ*	84.42	95.27	85.4	78.44	85.33	99.39	96.1	91.53	93.25	81.66	95.83	59.22	100.00	90.08	87.54	94.12	74.08	–	95.35	100.00	93.74	96.30	92.55	100.00	88.75	99.57	100.00	86.20
Δ⁺	89.88	94.05	94.64	94.05	89.81	83.33	95.00	91.67	88.89	88.67	83.33	85.19	100.00	90.48	86.3	97.22	76.67	–	84.44	100.00	88.79	94.44	89.29	100.00	90.00	88.89	100.00	89.68
TTD	719.05	752.38	757.14	752.38	808.33	250.00	475.00	366.67	266.67	433.33	333.33	851.85	300.00	723.81	862.96	388.89	460.00	–	506.67	200.00	976.67	377.78	714.29	300.00	450.00	266.67	300.00	627.78
STTD	304.35	182.82	139.95	182.82	312.93	555.56	113.89	162.04	246.91	266.67	277.78	410.15	0	246.6	373.94	38.58	288.89	–	424.69	0	303.58	154.32	321.71	0	233.33	246.91	0	224.24
Exergy	–	–	72.35	7.22	–	–	3.13	1.67	–	–	21.39	4.52	_	–	3416.39	1.95	–	–	59.59	2.48	–	–	6.33	2.30	–	–	3.55	16.16
Specific-Exerxy	–	–	179.68	69.52	–	–	146.37	1.82	–	–	122.61	50.9	–	–	230.27	220.86	–	–	59.13	321.82	–	–	202.32	145.61	–	–	222.38	175.20
B-IBI	–	–	2.00	1.67	–	–	1.67	2.33	–	–	2.00	1.33	–	–	4.00	2.67	–	–	2.33	2.33	–	–	2.33	3.00	–	–	2.33	1.67

Distinctness, the Feeding Structure Index (FSI) and the AMBI indices (Table 13). To be more precise, the Margalef Index and the Total Taxonomic Distinctness, which are highly correlated (r=+0.91; p<0.001) are only able to differentiate stations in the North arm from those in the South one. In his turn, AMBI can distinguish three types of 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 affected by dredging activities, and *c)* less disturbed stations of the North arm. These last two groups are also differentiated by the Feeding Structure Index.

Table 13

Discrimination between different areas in the Mondego estuary based on the values estimated for ecological indicators (Kruskal-Wallis test). FSI: Feeding Structure Index; TTD: Total Taxonomic Distinctness; N: Non dredged areas in the North arm; DA: Dredged area in the North arm; S: South arm areas with organic matter levels < 5%; OM: South arm areas with organic matter levels > 5%.

	AMBI	**FSI**	**Margalef**	**TTD**
	Average	Average	Average	Average
North arm-NDA	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-NDA, N, S 2-S-OM 3-OM	1-NDA, S, OM 2-N, S, OM	1-NDA, N 2-S, OM	1-NDA,S, OM 2-NDA,N, OM

The discrimination of different areas by AMBI is fundamentally due to the dominance of the 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 are prevalent, although species from group IV started appearing 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 B-IBI (Weisberg, 1997) (r=-0,61; p<0.01), the latter is not effective in discriminating the different areas. AMBI values also appeared negatively correlated with Specific Exergy (r=-0,67; p<0.05). This suggests that most of the information expressed by Specific Exergy was, in this case, very much related with the dominance of taxonomic groups usually absent in environmentally stressed situations.

It becomes clear that most of the sampling stations do not show differences when we account for the Average Taxonomic Distinctness values (Figure 10). In fact, even in the stations where just a few species were observed (*e.g.* E12 and E13 in 2000; E14 in 1998), the Average Taxonomic Distinctness measures present higher values, suggesting therefore high path length between species through the tree.

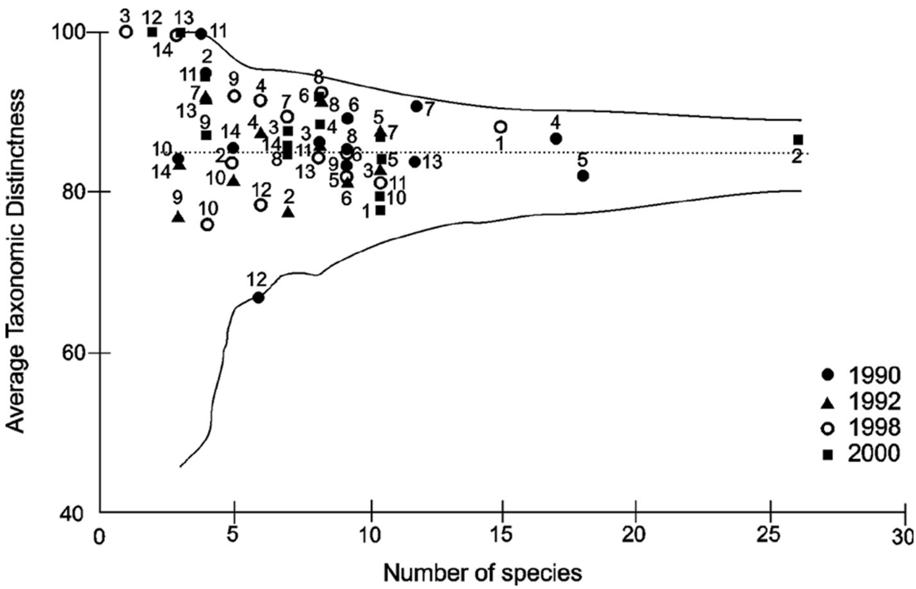

Figure 10. Confidence funnel (mean and 95% confidence interval) of the Average Taxonomic Distinctness in the Mondego estuary subtidal stations (numbers above the symbols correspond to station number) in 1990, 1992, 1998 and 2000.

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

Regarding the connexion between the physico-chemical 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 sensitive to the parameters normally associated to eutrophication (Table 14).

Table 14

Pearson correlations between the indicators' values and physico-chemical parameters in the Mondego estuary (subtidal communities). (*) = p<0.05.

	NO_2^-	NO_3^-	PO_4^{2-}	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

Let us now considerer the macrobenthic intertidal communities along the gradient of eutrophication symptoms in the south arm of the Mondego estuary. In this case, only the Total Taxonomic Distinctness (TTD) was able to significantly discriminate the three areas considered, exhibiting higher values at the *Zostera noltii* beds and the lowest one at the most eutrophic area (Table 15). Furthermore, the Margalef Index and the Exergy Index behaved as expected, showing higher values in the *Zostera noltii* area and lower values at the inner areas of the South arm, although they did not allow discriminating the intermediate eutrophic area from the most eutrophic one. On the contrary, the values estimated for several of the other indicators appear to indicate a better environmental status in the eutrophic area, which is inconsistent with our current knowledge of the system (Figure 11).

In this case, AMBI was unable to discriminate between the three areas, showing in all of them values close to 3, which indicates slightly polluted scenarios, *sensu* Borja *et al.* (2000), where species of the ecological group III dominate. Exceptionally, AMBI values between 4 and 5 were estimated from July to October (Figure 11), in the intermediate eutrophic area, which indicates a meanly polluted situation.

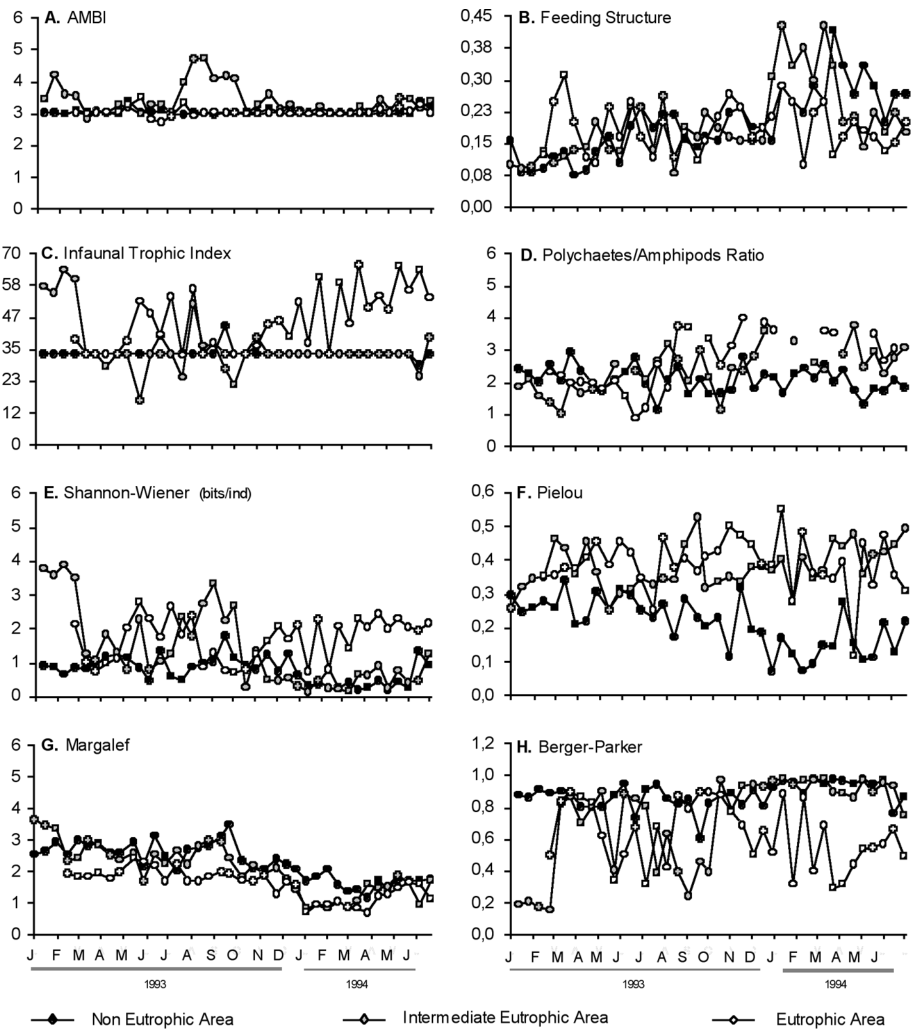

Figure 11. Temporal and spatial variation of the different ecological indicators applied to data on the intertidal communities of the South arm of the Mondego estuary.

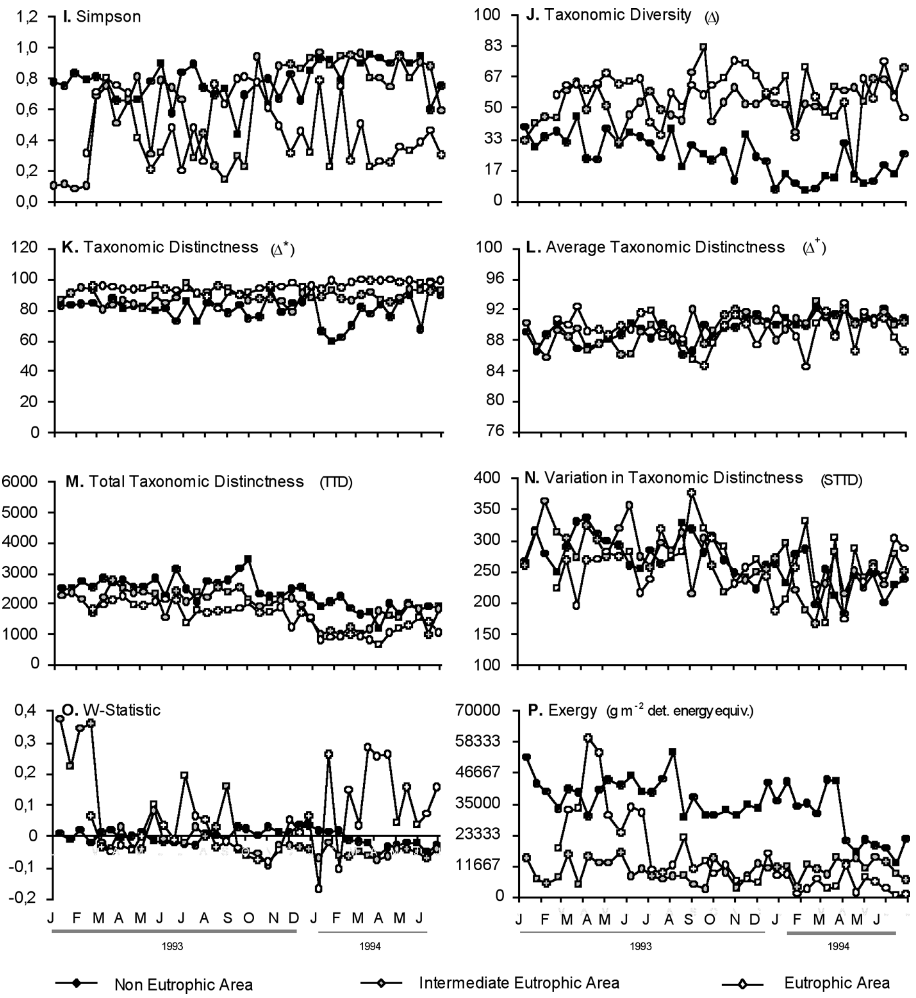

Figure 11. *(Continued)* - Temporal and spatial variation of the different ecological indicators applied to data on the intertidal communities of the South arm of the Mondego estuary.

The Polychaetes/Amphipods Ratio was able to illustrate the existing eutrophication gradient, exhibiting lower values in the *Zostera noltii* beds and higher values at the intermediate and most eutrophic areas, but was not sensitive enough to distinguish between these last ones ($p \leq 0.05$).

Table 15

Discrimination between different intertidal areas along a gradient of eutrophication symptoms in the South arm of the Mondego estuary, based on the values estimated for ecological indicators (Kruskal-Wallis test). TTD: Total Taxonomic Distinctness; NEA: Non Eutrophic Area; IA: Intermediate Eutrophic Area; EA: Eutrophic Area.

	TTD	**Exergy**	**Margalef**
	Average	Average	Average
Non Eutrophic Area	2348.68	35048.9	2.29
Intermediate Eutrophic Area	1919.39	10143.89	2.08
Eutrophic Area	1542.78	14893.58	1.60
GROUPS	1-NEA 2-IA 3-EA	1-NEA 2-IA,EA	1-NEA 2-IA, EA

Regarding correlations between ecological indicators' values and physico-chemical environmental factors, it is clear that the ITI index is highly correlated with the organic matter content in the sediment (r=-65; p<0.001), as actually Word (1990) verified in his work. Consequently, it presents the lowest values at the *Zostera noltii* area, where the organic matter content (M.O.%) in the sediment is higher (6.25% in average), which indicates organic enrichment, and the highest values in the eutrophic area, where the percentage of organic matter in the sediments is lower (3.25% in average). Therefore, it must be concluded that this indicator is not sensitive to the process of eutrophication, responsible for the occurrence of *Ulva* sp. blooms and the decrease of the *Zostera noltii* meadows, which is determined by the high concentration of nutrients in the water column.

Nevertheless, despite being capable to differentiate the *Zostera noltii* beds from the other areas in the South arm of the estuary, the Total Taxonomic Distincness, as well as the Margalef, Pielou, and the Exergy based indices are positively and significantly correlated with the organic matter content in the sediments (Table 16). Moreover, the Exergy and the Margalef indices are negatively correlated, respectively, with the ammonium and nitrite concentrations in the water column (Exergy against the ammonium concentration: r=-0.30, $p<0.05$; Margalef Index against the Nitrites concentration: r=-0.25, $p<0.05$). They show, therefore, sensitive to the fact that, in this case, the benthic communities' structure is strongly influenced by the water column nutrients concentration, but not by the organic matter content present in the sediments.

Table 16

Pearson correlations between ecological indicators' values and the organic matter content in the sediments for the intertidal communities of the Mondego estuary. TTD: Total Taxonomic Distinctness. (*): $p<0.05$; (**): $p<0.001$.

	TTD	Margalef	Pielou	Exergy
Organic Matter Content (%)	-0.76**	-0.64*	-0.59*	-0.62*

4.3.2. The Mira estuary

Regarding the Mira estuary, the absence of environmental data did not allow to establish any relation between ecological indicators' values and physico-chemical parameters. In addition, we could not apply analysis of variance to assess the performance of the different indicators in the different areas of the estuary, due to the fact that we could not set up any zonation criteria. We could confirm this through the application of MDS analysis (Figure 12).

Actually, Andrade (1986) already mentioned the impossibility of distinguishing contrasting areas, considering the Mira estuary as a continuum. However, Pearson correlations between the different ecological indicators applied show interesting results (Table 17).

First, as expected, we observe significant correlations between the values of the Shannon-Wiener, Margalef, Simpson, Berger-Parker, and Pielou indices.

Furthermore, Taxonomic Diversity is also highly correlated with the Shannon-Wiener, Pielou and Simpson indices. This last correlation is expected to occur when the taxonomic tree collapses to a single level hierarchy (all species in the same genus), as in that case, taxonomic diversity becomes a form of Simpson diversity (Warwick & Clarke, 1994).

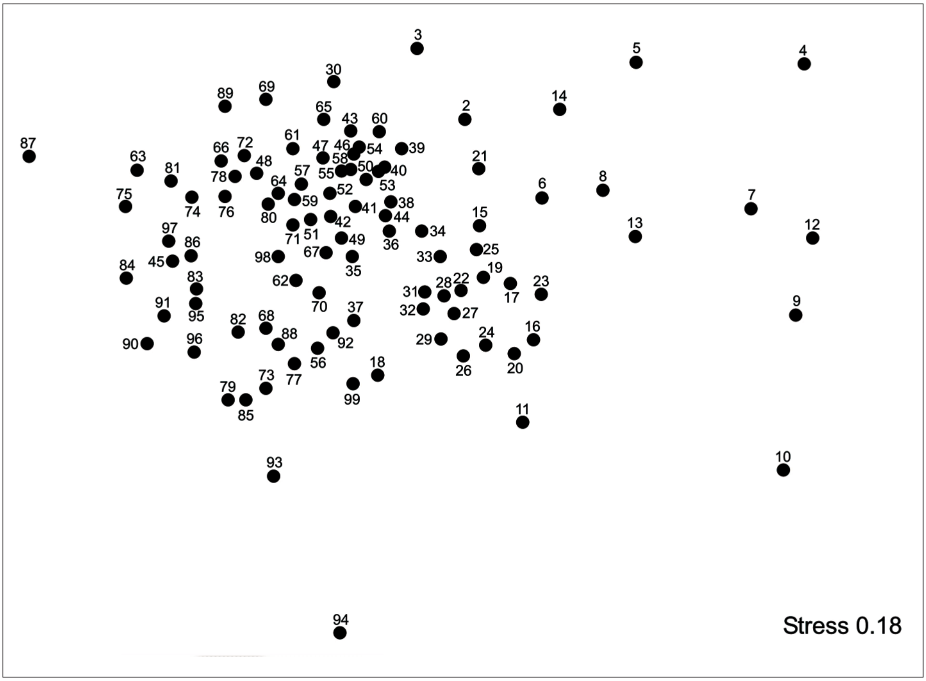

Figure 12. Mira estuary. Two-dimensional MDS plot of taxa abundance (Clarke, 1993; Clarke & Warwick, 1994). (numbers above the symbols correspond to station number).

On the other hand, Total Taxonomic Distinctness appears strongly correlated with the Margalef Index, as observed in the Mondego estuary. In fact, these two indices are based on species richness, and thus Total Taxonomic Distinctness is supposed to be capable of differentiating when an assemblage consisting of closely-related species is less rich than one composed of distantly related species. However, such high correlation lead us to think that this is a case of an analogue performance of both indices,

Table 17

Pearson correlations between the values of the different ecological indicators estimated based on data proceeding from sampling stations in the Mira estuary. Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ+: Average Taxonomic Distinctness (presence/absence of species); FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness; P/A: Polychaetes/Amphipods Ratio. (*): $p \leq 0.05$; (**): $p \leq 0.01$.

	AMBI	Berger	Δ+	Δ*	Δ	FSI	ITI	STTD	Margalef	Pielou	P/A	TTD	Shannon
Berger	0.20												
Δ+	-0.08	0.03											
Δ*	-0.24	-0.22	0.60*										
Δ	-0.24	-0.96*	0.12	0.43									
FSI	-0.51	0.06	-0.06	-0.06	-0.05								
ITI	0.03	0.24*	0.16	0.28*	-0.15	-0.01							
STTD	-0.22	-0.13	-0.48	-0.33*	0.07	0.21	-0.13						
Margalef	-0.10	-0.24	-0.18	-0.27*	0.19	0.31	-0.29	0.34*					
Pielou	-0.14	-0.88*	0.02	0.33*	0.89*	-0.13	-0.14	-0.09	-0.07				
P/A	0.20	0.28	-0.09	-0.54*	-0.36*	0.07	-0.38	0.16	0.47*	-0.51*			
TTD	0.02	0.02	-0.13	-0.36*	-0.07	0.27	-0.23	0.30*	0.93*	-0.35*	0.66*		
Shannon	-0.24	-0.88*	-0.04	0.15	0.87*	0.12	-0.24*	0.27*	0.58*	0.66*	-0.10	0.34*	
Simpson	0.21	0.98*	0.02	-0.25	-0.98*	0.02	0.23	-0.15	-0.27	-0.89*	0.30	0.00	-0.91*

and thus that, in this case study, Total Taxonomic Distinctness cannot be considered better than the Margalef index when measuring diversity.

It is also intriguing to find out that AMBI is correlated with Taxonomic Diversity, but not with any other diversity index. In fact, AMBI shows in all cases values below 2.5, which indicates a situation of good ecological status in all the stations (Table 18). The same way, as it is shown in Figure 13, the comparison of samples with the master list (TAXDTEST) showed that the Average Taxonomic Distinctness was, in most of the stations, within the 95% confidence intervals of the probability funnel for all samples, indicating also a good ecological status *sensu* Somerfield *et al.* (2003). These results match the assessment found in a number of previous studies (*e.g.* Raposo *et al.*, 1996; Costa *et al.* 1994), which consider the Mira estuary as representing what a pristine system should be.

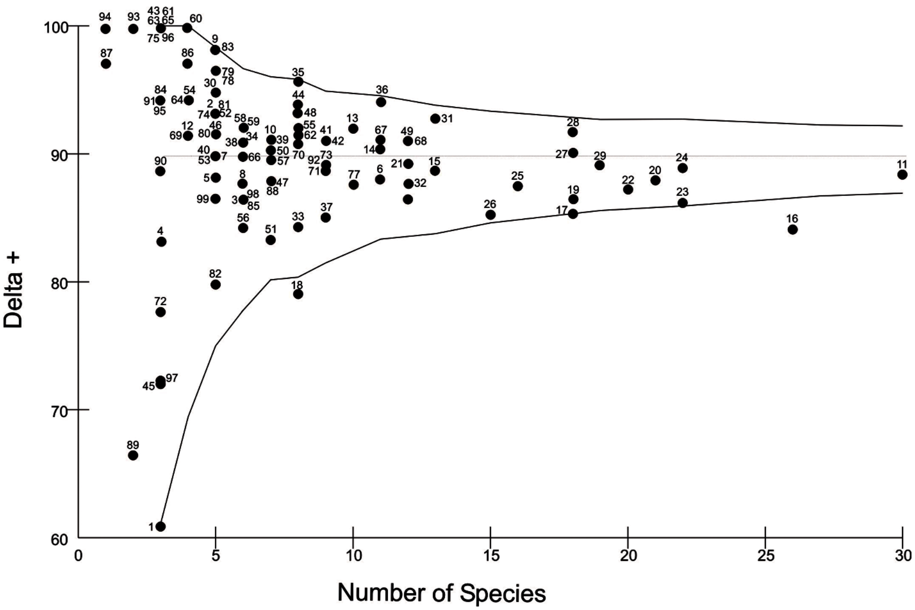

Figure 13. Departure from the theoretically expected Average Taxonomic Distinctness and 95% confidence funnel of all individual samples using presence/absence data for the Mira estuary. (numbers above the symbols correspond to station number).

Table 18

Indices values estimated based on data from each sampling station at the Mira estuary. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods Ratio; Δ: Taxonomic Diversity; Δ^*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	AMBI	FSI	ITI	P/A	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ^*	Δ^+	TTD	STTD
S1	1.29	0	33.34	–	1.06	0.67	0.67	0.75	0.57	22.48	52.56	61.11	183.33	432.10
S2	1.69	0.33	57.15	–	2.16	0.93	1.34	0.38	0.21	71.27	90.28	93.33	466.67	177.78
S3	1.05	0	33.34	–	2.37	0.92	1.55	0.30	0.19	70.83	87.18	86.67	520.00	340.74
S4	1.50	0	33.34	0.48	1.58	1.00	0.99	0.33	0.23	64.10	83.33	83.33	250.00	555.56
S5	1.75	0	33.34	–	2.25	0.97	1.61	0.33	0.15	72.73	85.71	88.33	441.67	336.11
S6	1.35	0.67	46.18	0.50	2.96	0.86	2.16	0.21	0.14	76.12	88.92	88.18	970.00	390.63
S7	0.12	2.00	57.15	–	1.87	0.81	1.13	0.43	0.31	51.65	74.49	90.00	450.00	233.33
S8	0.43	1.50	42.34	1.04	1.59	0.61	1.06	0.67	0.47	45.84	86.96	87.78	526.67	424.69
S9	0.68	0.25	60.61	–	2.12	0.91	1.21	0.36	0.23	74.90	97.04	98.33	491.67	25.00
S10	0	1.50	74.36	0	2.41	0.86	1.63	0.38	0.21	71.85	91.50	91.27	638.89	228.02
S11	1.31	0.50	58.73	0.94	4.04	0.82	5.50	0.24	0.1	77.82	86.39	88.58	2657.47	323.02
S12	0	1.00	35.56	–	1.20	0.60	0.62	0.62	0.5	49.08	97.24	91.67	366.67	347.22
S13	0.17	1.50	62.42	–	2.45	0.74	1.86	0.46	0.27	56.36	76.71	92.22	9222.22	254.32
S14	0.19	0.25	33.34	0.88	1.49	0.43	1.82	0.76	0.59	39.71	96.76	90.61	996.67	330.95
S15	2.71	0.20	45.37	1.60	2.69	0.73	2.07	0.33	0.22	72.40	92.62	88.89	1155.56	318.14
S16	2.68	0.63	67.44	2.28	2.34	0.50	3.78	0.61	0.4	49.30	81.78	84.26	2190.67	460.69
S17	2.35	0.50	58.91	2.42	1.82	0.44	2.47	0.71	0.52	38.62	80.12	85.51	1539.22	311.16
S18	2.67	1.00	78.85	–	2.26	0.75	1.26	0.34	0.24	64.54	85.20	79.17	633.33	508.43
S19	2.33	0.42	64.35	1.25	3.10	0.74	3.16	0.31	0.17	66.37	80.08	86.71	1560.78	473.35
S20	2.68	0.46	96.67	–	1.48	0.34	2.80	0.77	0.6	32.83	82.73	88.17	1851.67	382.65

Table 18 ***(Continued)***

Indices values estimated based on data from each sampling station at the Mira estuary. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods Ratio; Δ: Taxonomic Diversity; Δ^*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	AMBI	FSI	ITI	P/A	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ^*	Δ^+	TTD	STTD
S21	1.56	0.20	33.34	–	2.67	0.74	2.36	0.36	0.22	68.01	87.00	87.88	1054.55	374.96
S22	2.22	0.11	55.78	0.83	2.45	0.57	2.65	0.53	0.32	59.23	86.59	87.37	1747.37	445.71
S23	2.54	0.83	63.62	2.28	2.24	0.50	3.29	0.64	0.43	49.07	85.60	86.36	1900.00	426.12
S24	2.85	0.43	66.67	3.00	0.96	0.22	2.66	0.88	0.77	19.47	84.84	89.11	1960.32	262.50
S25	2.70	0.60	66.67	–	1.18	0.29	2.18	0.83	0.69	23.02	73.56	87.64	1402.22	418.96
S26	2.72	0.25	66.67	–	0.60	0.15	1.62	0.90	0.81	13.00	69.30	85.40	1280.95	358.18
S27	2.85	0.20	66.67	2.58	0.52	0.12	1.94	0.93	0.87	10.08	76.37	90.31	1625.49	292.72
S28	2.72	0.13	66.67	2.34	0.84	0.20	2.07	0.88	0.77	16.66	72.81	91.94	1654.90	254.56
S29	2.78	0.20	66.67	2.57	0.69	0.16	2.18	0.91	0.83	13.27	76.61	89.28	1696.30	300.91
S30	1.20	0.25	62.82	–	2.13	0.92	0.90	0.32	0.23	75.95	98.95	95.00	475.00	225.00
S31	2.65	0.18	66.67	2.44	0.79	0.21	1.46	0.87	0.76	17.44	72.56	92.95	1208.33	203.13
S32	2.80	0	66.67	2.92	0.68	0.19	1.39	0.90	0.82	13.38	73.08	86.62	1039.39	355.38
S33	2.57	0	60.56	2.65	0.98	0.33	0.96	0.81	0.67	22.70	67.96	84.52	676.19	415.25
S34	1.82	0	65.51	1.96	1.84	0.71	0.84	0.51	0.34	56.33	85.38	91.11	546.67	217.28
S35	0.92	0.33	86.36	1.49	1.94	0.65	1.13	0.46	0.32	66.69	98.75	95.83	766.67	111.61
S36	1.50	0.38	58.49	0.60	2.85	0.82	2.05	0.38	0.19	74.93	92.86	94.24	1036.67	143.62
S37	1.89	0.29	92.93	0.11	2.42	0.76	1.56	0.36	0.23	67.52	88.13	85.19	766.67	582.99
S38	1.54	0	57.80	1.53	2.17	0.84	1.01	0.36	0.24	62.31	82.30	91.11	546.67	217.28
S39	1.55	0.75	37.85	1.27	0.60	0.21	0.99	0.92	0.84	14.70	92.25	90.48	633.33	306.12
S40	2.29	0	66.97	0.34	1.71	0.74	0.85	0.44	0.35	60.92	93.82	90.00	450.00	233.33

Table 18 *(Continued)*

Indices values estimated based on data from each sampling station at the Mira estuary. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods Ratio; Δ: Taxonomic Diversity; Δ^*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	AMBI	FSI	ITI	P/A	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ^*	Δ^+	TTD	STTD
S41	1.38	0	61.17	0.66	2.51	0.79	1.58	0.37	0.23	71.34	92.80	91.20	820.83	254.42
S42	2.66	0.13	69.98	1.36	2.65	0.83	1.58	0.37	0.21	71.93	90.49	91.20	820.83	300.71
S43	2.63	0	89.82	0.08	0.88	0.55	0.37	0.80	0.66	33.58	100.00	100.00	300.00	0
S44	0.89	0.60	76.49	–	2.65	0.88	1.50	0.31	0.18	76.50	93.27	94.05	752.38	143.14
S45	3.00	0	37.19	–	1.41	0.89	0.61	0.50	0.38	50.87	82.46	72.22	216.67	1543.21
S46	2.23	0	73.94	0.21	1.43	0.62	0.80	0.58	0.44	53.29	95.89	91.67	458.33	291.67
S47	2.13	0.17	73.50	0.23	2.06	0.73	1.34	0.42	0.3	64.50	92.49	88.10	616.67	506.42
S48	2.37	0.14	54.50	0.78	2.65	0.88	1.69	0.37	0.19	71.96	88.77	93.45	747.62	224.99
S49	1.83	0.20	77.28	0.52	3.03	0.85	2.05	0.25	0.15	80.91	95.39	91.16	1093.94	296.46
S50	1.76	0	63.69	0.37	2.37	0.85	1.49	0.35	0.23	72.32	93.33	90.48	633.33	253.21
S51	2.61	0	61.42	0.37	2.36	0.84	1.24	0.37	0.23	67.36	87.73	83.33	583.33	529.10
S52	2.55	0	90.79	0.80	1.30	0.56	0.67	0.74	0.56	42.69	97.35	93.33	466.67	177.78
S53	1.70	0	78.01	0.30	2.16	0.93	0.80	0.39	0.25	72.08	95.59	90.00	450.00	233.33
S54	2.18	0	83.33	0.12	1.44	0.72	0.61	0.62	0.44	55.35	99.52	94.44	377.78	154.32
S55	2.29	0.14	91.35	0.05	1.65	0.55	1.25	0.66	0.47	50.51	95.57	92.26	738.10	227.82
S56	1.15	0.20	84.33	0.36	1.97	0.76	0.94	0.47	0.31	66.38	96.42	84.44	506.67	720.99
S57	2.65	0	60.26	0.34	2.26	0.81	1.19	0.38	0.25	66.70	88.73	89.68	627.78	330.06
S58	2.35	0	82.93	0.14	1.63	0.63	1.04	0.65	0.45	53.07	97.12	92.22	553.33	180.25
S59	2.14	0	45.90	0.73	2.13	0.82	1.18	0.48	0.28	63.49	88.46	92.22	533.33	217.28
S60	2.00	0	50.01	0.60	1.73	0.86	0.81	0.50	0.33	66.95	100.00	100.00	400.00	0

Table 18 ***(Continued)***

Indices values estimated based on data from each sampling station at the Mira estuary. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods Ratio; Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	AMBI	FSI	ITI	P/A	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ*	Δ^+	TTD	STTD
S61	2.51	0.50	96.52	0.03	1.11	0.70	0.41	0.67	0.53	46.98	100.00	100.00	300.00	0
S62	1.71	0.33	86.97	0.36	1.66	0.55	1.17	0.71	0.51	47.77	97.68	91.67	733.33	228.17
S63	3.00	0	57.54	–	1.08	0.68	0.40	0.62	0.51	49.11	100.00	100.00	300.00	0
S64	1.85	0.33	98.73	0.02	0.92	0.46	0.48	0.75	0.62	38.21	99.75	94.44	377.78	154.32
S65	2.60	0	90.67	0.07	0.91	0.57	0.51	0.80	0.66	34.47	100.00	100.00	300.00	0
S66	2.98	0	55.65	1.04	1.48	0.57	0.89	0.60	0.45	53.64	98.33	90.00	540.00	325.93
S67	1.50	0.1	79.29	0.73	2.05	0.59	1.96	0.57	0.38	60.19	96.79	91.21	1003.33	321.76
S68	1.03	0.38	81.60	0.09	2.46	0.69	2.09	0.40	0.26	69.66	94.46	89.39	1072.73	333.64
S99	2.67	0.33	93.10	0.07	1.45	0.72	0.88	0.67	0.46	52.36	97.62	91.67	366.67	374.22
S70	1.57	0.33	98.62	0.35	0.53	0.18	1.02	0.93	0.89	12.51	92.59	91.07	728.57	247.66
S71	2.56	0.13	64.78	0.41	2.89	0.91	1.98	0.29	0.15	74.79	87.74	88.89	800.00	416.67
S72	3.00	0	87.88	0	0.92	0.58	0.57	0.80	0.65	26.46	75.49	77.78	233.33	61.73
S73	1.83	0.50	90.50	0.06	2.07	0.65	1.21	0.38	0.28	64.56	90.21	89.35	804.17	264.70
S74	3.00	0	59.84	1.08	1.79	0.77	0.90	0.44	0.33	66.18	99.19	93.33	466.67	122.22
S75	3.00	0	68.26	–	1.31	0.83	0.37	0.52	0.44	56.45	100.00	100.00	300.00	0
S76	2.56	0.50	89.30	0	2.15	0.83	1.14	0.46	0.28	67.16	92.72	90.00	540.00	214.81
S77	1.75	0.25	91.67	0.48	1.67	0.50	1.37	0.69	0.5	42.85	85.00	87.78	877.78	474.07
S78	3.00	0	77.68	0.41	1.81	0.78	0.85	0.44	0.32	67.29	98.85	95.00	475.00	113.89
S79	1.50	0.67	94.49	0.10	0.87	0.38	0.71	0.85	0.73	25.39	95.47	95.00	475.00	113.89
S80	2.67	0	70.00	0.37	2.20	0.95	1.18	0.33	0.21	71.52	90.32	91.67	458.33	291.67

Table 18 *(Continued)*

Indices values estimated based on data from each sampling station at the Mira estuary. FSI: Feeding Structure Index; ITI: Infaunal Trophic Index; P/A: Polychaetes/Amphipods Ratio; Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ^{+}: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness.

	AMBI	FSI	ITI	P/A	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ*	Δ^{+}	TTD	STTD
S81	2.77	0	64.34	0.54	1.91	0.82	1.06	0.38	0.3	69.45	98.85	93.33	466.67	122.22
S82	1.52	0.25	95.71	0	0.64	0.27	0.75	0.90	0.82	17.74	98.73	80.00	400.00	377.78
S83	1.66	0.25	95.10	–	0.69	0.30	0.70	0.89	0.8	18.90	95.12	96.67	483.33	44.44
S84	3.00	0	88.60	0.06	0.81	0.51	0.46	0.83	0.7	29.05	95.99	94.44	283.33	61.73
S85	1.56	0.50	97.46	0.43	0.94	0.36	0.74	0.84	0.72	19.18	67.75	86.67	520.00	785.19
S86	3.00	0	61.73	–	1.91	0.95	0.91	0.37	0.25	72.51	97.10	97.22	388.89	38.58
S87	3.00	0	100.00	–	0		0	1.00	1.00	0	0	0	0	0
S88	1.78	0.17	82.18	0.32	2.10	0.75	1.18	0.52	0.32	65.44	96.29	88.10	616.67	479.97
S89	3.00	0	66.67	0	1.00	1.00	0.53	0.5	0.41	39.22	66.67	66.67	133.33	0
S90	2.14	0.50	76.67	0	1.38	0.87	0.64	0.57	0.40	56.86	95.24	88.89	266.67	246.91
S91	1.65	0.50	93.94	–	0.57	0.36	0.48	0.9	0.81	18.70	99.55	94.44	283.33	61.73
S92	2.19	0.13	79.78	0.69	2.05	0.65	1.35	0.4	0.32	66.25	97.11	89.35	804.17	372.73
S93	1.50	0	66.67	0.30	1	1	0.53	0.5	0.41	58.82	100.00	100	200	0
S94	1.50	–	100.00	–	0	–	0	1	1.00	0	0	0	0	0
S95	3.00	0	37.19	–	1.06	0.67	0.61	0.75	0.58	38.96	92.31	94.44	283.33	61.73
S96	2.50	0.50	66.67	–	1.58	1.00	0.67	0.33	0.30	70.18	100.00	100.00	300.00	0
S97	2.40	0	33.34	–	1.52	0.96	0.71	0.40	0.32	51.06	75.00	72.22	216.67	246.91
S98	3.00	0	40.69	0.20	1.08	0.42	0.93	0.81	0.67	28.52	85.99	86.67	520.00	266.67
S99	2.97	0.25	42.93	0.05	1.37	0.59	0.63	0.60	0.46	40.69	75.86	86.67	433.33	322.22

4.3.3. The Mar Menor lagoon

The different environmental parameters' values analysed showed that the areas mostly affected by organic enrichment correspond to stations M2 and M6, where organic matter content in sediments reaches values higher than 5%. These stations also have in common the dominance of polychaetes, being *Heteromastus filiformis* the most abundant specie. We should, therefore, expect the occurrence of lower values of Exergy and Specific Exergy, taxonomic diversity measures, and W-Statistic, as well as higher values regarding AMBI, the Polychaetes/Amphipods Index, Feeding Structure Index, 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, Margalef Index, Total Taxonomic Distinctness and AMBI indicated disturbance (Table 19).

Moreover, the Margalef Index and Total Taxonomic Distinctness are the only indicators capable of detecting significant differences between organically enriched and non enriched areas ($p<0.05$) (Table 20). The AMBI values are similar in all sampling stations, indicating moderate disturbance in M2 and M6 and slight disturbance in the other ones. These results show that AMBI did not allow to distinguish between different disturbance intensities in the study area. Nevertheless, AMBI presents a positive response, although not significant ($r=+0.41$; $p>0.05$), relatively to the organic matter content in the sediments.

Table 20

Indices values able to significantly discriminate (One-way ANOVA) organically enriched from non organically enriched areas in the Mar Menor lagoon.

	Margalef	Total Taxonomic Distinctness
	Average	Average
Organically enriched areas	1.28	585.94
Non organically enriched areas	3.92	1846.59

In his turn, the W-Statistic gives rather confusing results as station M2, which present organic matter contents in the sediments lower than M6, appear as the most polluted one (W=-0.3).

Table 19

Indices values estimated at the different sampling stations (M1 to M10) in Mar Menor lagoon in A: July, B: November, C: February and D: May. 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.

	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	2885503836	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	183671203	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	546460384	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	192624681	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	15762446	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		14126661509	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	899957796	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	76867912	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	145227127	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	1555244	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	3249672701	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	70381987	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	2523455	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	28713101	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	301455400	70064

In general terms, a similar pattern of variation 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, such as salinity or, in the case of Margalef and Shannon-Wiener indices, also with sediment particles size. Specific Exergy showed a clear positive correlation with the presence of certain heavy metals as Pb (r=+0.89; $p\leq0.05$) and Zn (r=+0.71; $p\leq0.05$), which is not what we could 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 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 variatons in the system biomass quality (β factors). In the case of Specific Exergy, the influence of biomass variations is much less important, as changes in β factors related to the biomass quality play a major role. In this sense, the decline of taxonomic groups affected by toxic substances, as a function of different degrees of tolerance, should be clearly reflected in Specific Exergy values. Nevertheless, Molluscs, namely Bivalves, are known by their ability to bio-accumulate heavy metals, contrarily to what happens, for instance, with Polychaetes, Crustaceans and Echinoderms. Since the β factor estimated for Molluscs is higher than for the other mentioned groups (see Table 9), it becomes straightforward to understand why Specific Exergy values were found to be higher in areas affected by heavy metals pollution.

4.3.4. Escombreras basin

In this study site, two criteria were established *a priori* to separate the stations in two groups, and to test the different ecological indicators discriminatory capability. Firstly, the concentration of organic matter in the sediment was taken into account. According to this criteria, stations E2, E7 and E8 were considered as organically enriched. Secondly, MDS analysis was applied to data on taxa abundance in order to separate the stations into different groups (Figure 14).

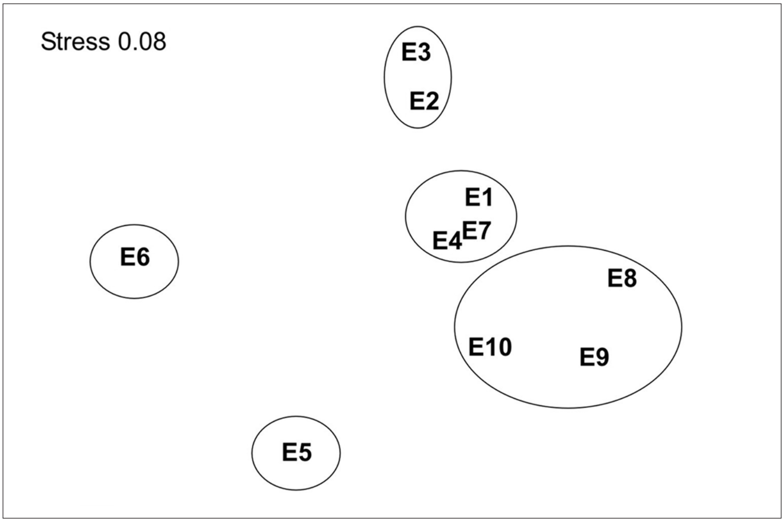

Figure 14. MDS analysis. Two-dimensional plot of stations based on taxa abundance in the Escombreras basin.

In none of the cases the indicators showed to be able 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 21).

Other indicators, for instance the W-Statistic, mostly show a performance similar to diversity measures based on species abundance. On the other hand, AMBI indicates that station E1 is the most polluted one, due to

Table 21

Values of ecological indicators estimated at the different sampling stations (E1 to E10) at Escombreras basin. 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.

	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

the dominance of *Polydora ciliata*, a polychaete belonging to ecological group IV, which is referred by several authors (*i.e.* Pearson & Rosenberg, 1978; Gray, 1979; Villalba & Vietiez, 1985) as indicator of organic and oil pollution.

Finally, the performance of indicators based on ecological strategies was very dissimilar 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 physico-chemical environmental parameters.

4.3.5. 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 placement of the floating cages, were considered as representing a pristine situation, in opposition to the other sampling periods. Values estimated for the different indicators are given in Table 22.

In the first case, only AMBI was able to distinguish between reference and disturbed stations ($p<0.05$), in spite of the fact that only one group of polychaetes has been considered. Nevertheless, such differentiation, although statistically significant, showed to be irrelevant in terms of differentiating ecological status, as in general, all the stations are identified as «good» *sensu* Borja *et al.* (2000).

As for the comparison of samples from August 1996 with samples from posterior dates, none of the indices was able to illustrate the *a priori* assumed pristine situation, neither to distinguish it from the subsequent periods.

Again, significant correlations are found between diversity measures, Taxonomic Diversity, and the W-Statistic, and also between the Margalef Index and Total Taxonomic Distinctness (Table 23).

Table 22

Indices values estimated at the different sampling stations (J1 to J10) in Cape Tiñoso. ITI: Infaunal Trophic Index; Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness; A96: August 1996; N96: November 1996; F97: February 1997; J97: June 1997; N97: November 1997.

		AMBI	ITI	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ*	Δ⁺	TTD	STTD	W-Statistic
J1	A96	1.5	66.67	0.65	0.65	0.56	0.83	0.67	22.22	66.67		133.33	0	0.33
	N96	1.32	50.99	2.54	0.9	2.12	2.27	0.15	49.02	57.47	56.35	394.44	171.33	0.33
	F97	-	-	-	-	-	-	-	-	-	-	-	-	-
	J97	0.62	56.49	2.96	0.86	2.79	0.25	0.14	56.19	65.31	62.42	686.67	143.62	0.21
	N97	1.43	56.95	2.52	0.84	2.2	0.25	0.18	49.58	60.55	58.93	471.43	148.46	0.25
J2	A96	0.94	63.89	2.92	0.84	3.11	0.28	0.14	52.66	62.11	64.44	644.44	69.14	0.37
	N96	1.6	58.34	2.6	0.93	2.16	0.38	0.13	51.53	59.46	61.9	433.33	109.6	0.35
	F97	-	-	-	-	-	-	-	-	-	-	-	-	-
	J97	-	-	-	-	-	-	-	-	-	-	-	-	-
	N97	1.5	64.92	1.61	1.62	1.7	0.68	0.46	31.87	59.24	61.11	366.67	98.77	0.06
J3	A96	2.27	53.54	3.09	0.86	3.15	0.28	0.13	53.82	61.51	62.63	751.52	143.61	0.3
	N96	2.19	41.03	3.03	0.95	3.12	0.23	0.06	56.84	60.73	62.5	562.5	52.08	0.43
	F97	1.8	60	1.92	0.96	1.86	0.42	0.1	45	50	52.78	211.11	316.36	0.31
	J97	1.5	62.97	2.64	0.94	2.73	0.37	0.08	56.94	62.12	62.7	438.89	76.85	0.74
	N97	1.7	43.95	3.17	0.88	3.56	0.31	0.11	53.32	60.08	60.61	727.27	123.2	0.43
J4	A96	1.4	58.49	2.03	0.83	2.3	0.26	0.32	33.24	53.21	59.31	189.97	65.73	0.23
	N96	1.87	45.84	1.75	0.88	1.44	0.33	0.25	36.31	48.41	47.22	188.89	38.58	0.4
	F97	2.36	33.34	2.13	0.92	2.06	0.42	0.14	50	58.33	61.67	308.33	225	0.31
	J97	1.43	49.28	2.91	0.88	2.87	0.29	0.13	49.08	56.44	61.11	611.11	111.11	0.53
	N97	1.68	51.29	3.34	0.88	3.99	0.26	0.1	54.67	60.84	62.09	869.23	85.87	0.32
J5	A96	1.55	65.48	1.39	0.75	1.98	0.48	0.52	42.32	43.23	58.74	135.64	123.54	0.33
	N96	1.5	33.34	0.92	0.92	0.91	1	0.33	44.44	66.67	66.67	133.33	0	-
	F97	1.07	57.15	2.52	0.98	2.57	0.16	0.05	61.11	64.17	63.33	380	44.44	0.83
	J97	1.61	52.85	3.81	0.91	4.58	0.21	0.07	54.53	58.91	61.15	1039.58	151.37	0.48
	N97	1.12	50.01	0.81	0.81	0.72	0.5	0.5	33.33	66.67	66.67	133.33	0	0.46

Table 22 *(Continued)*

Indices values estimated at the different sampling stations (J1 to J10) in Cape Tiñoso. ITI: Infaunal Trophic Index; Δ: Taxonomic Diversity; Δ*: Taxonomic Distinctness; Δ+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness; A96: August 1996; N96: November 1996; F97: February 1997; J97: June 1997; N97: November 1997.

		AMBI	ITI	Shannon	Pielou	Margalef	Berger	Simpson	Δ	Δ*	$Δ^+$	TTD	STTD	W-Statistic
	A96	1.78	62.02	2.33	0.78	1.76	0.33	0.23	46.59	60.57	59.52	476.19	187.07	0.25
	N96	1.5	55.56	2.28	0.88	2.28	0.4	0.17	43.52	52.22	54.44	326.67	239.51	0.37
J6	F97	1.16	59.26	3.17	1	3.64	0.12	0	61.11	61.11	61.11	550	123.46	0.94
	J97	1.96	51.29	2.93	0.92	3.12	0.3	0.09	55.56	61.03	60.65	545.83	125.81	0.018
	N97	1.5	42.86	2.99	0.94	3.03	0.2	0.08	47.25	51.19	52.78	475	208.33	0.53
	A96	1.39	66.67	1.51	0.54	1.78	0.72	0.52	26.94	61.72	61.11	366.67	135.8	0.09
	N96	1.65	60	1.77	0.76	1.74	0.6	0.33	34.44	51.67	55	275	113.89	0.24
J7	F97	1.15	70.59	3.18	0.96	3.18	0.18	0.07	60.42	64.7	64.7	640.74	48.83	0.53
	J97	1.75	63.07	3.95	0.9	5.54	0.18	0.06	59.01	63.08	61.59	1293.33	103.83	0.6
	N97	1.68	62.97	2.28	0.88	2.28	0.5	0.17	46.76	56.11	58.89	353.33	106.17	0.23
	A96	1.67	63.89	1.3	0.5	1.33	0.74	0.57	17.53	40.49	61.11	366.67	172.84	-0.08
	N96	2	38.9	1.46	0.92	1.12	0.5	0.27	42.22	57.58	61.11	183.33	61.73	0.47
J8	F97	1.16	62.97	2.73	0.97	2.73	0.2	0.06	61.57	65.2	65.08	455.56	23.94	0.35
	J97	1.64	57.15	3.5	0.95	3.94	0.19	0.06	58.73	62.29	63.03	819.44	75.84	0.56
	N97	-	-	-	-	-	-	-	-	-	-	-	-	-
	A96	-	-	-	-	-	-	-	-	-	-	-	-	-
	N96	1.5	38.1	1.38	0.87	1.03	0.5	0.33	41.27	61.9	61.11	183.33	61.73	0.13
J9	F97	1.16	66.66	2.06	0.89	1.82	0.44	0.19	47.69	59.2	60	300	122.22	0.05
	J97	1.74	56.41	3.58	0.94	3.99	0.18	0.06	58.31	61.93	61.9	866.67	93.32	0.43
	N97	1.6	44.45	2.46	0.82	2.58	1	0.21	48.89	61.85	61.9	495.24	116.21	0.52
	A96	-	-	-	-	-	-	-	-	-	-	-	-	-
	N96	-	-	-	-	-	-	-	-	-	-	-	-	-
J10	F97	-	-	-	-	-	-	-	-	-	-	-	-	-
	J97	1.98	41.39	3.73	0.95	4.16	0.21	0.05	55.34	58.35	58.89	883.33	111.46	0.27
	N97	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 23

Pearson correlations between the values of the different ecological indicators estimated based on data proceeding from sampling stations at Cape Tiñoso. Δ: Taxonomic Diversity; Δ^*: Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness; STTD: Variation in Taxonomic Distinctness (*): $p \leq 0.05$; (**): $p \leq 0.01$.

	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*

On the other hand, regarding the response of ecological indicators to environmental parameters, contrarily to what should be expected, a positive correlation between several diversity measures, Taxonomic Diversity, Total Taxonomic Distinctness, and the concentration of chlorophyll *a* in the water column was found (Table 24). These results suggest that the instalation of floating cages assigned to red tuna fattening, at least in the first and a half year, had fairly small environmental impact, determining an intermediate disturbance situation, which in fact favoured an increase in diversity.

Table 24

Pearson correlations between the values of the different ecological indicators estimated based on data proceeding from sampling stations at Cape Tiñoso. Δ: Taxonomic Diversity; Δ^+: Average Taxonomic Distinctness; TTD: Total Taxonomic Distinctness (*): $p \leq 0.05$; (**): $p \leq 0.01$.

	Shannon	Simpson	Berger	Pielou	Δ	Margalef	TTD	Δ^+
Chlorophyll *a*	0.46**	-0.39*	-0.41*	0.31*	0.37*	0.48**	0.38*	-0.23

The Average Taxonomic Distinctness is the exception, showing negative correlation (although not significant) with the concentration of chlorophyll *a* in the water column. This confirms the fact that the Average Taxonomic Distinctness response is monotonic, contrarily to other diversity measures, as said already by Warwick & Clarke (1994).

4.4. Was the ecological indicators performance satisfactory in the case studies?

4.4.1. Indices based on indicator species

In general, AMBI worked reasonably well, being able to discriminate areas under pressure accounting for the benthic subtidal communities in the

Mondego estuary, Mar Menor, and Cape Tiñoso. It was, however, inefficient in differentiating areas with clearly different eutrophication symptoms along a spatial gradient in the south arm of the Mondego estuary (*e.g.* dominance of *Zostera noltii vs. Ulva* sp. as main primary producers). In the case of the Mondego estuary, this may perhaps be explained if we accept that eutrophication effects, which are clearly visible at the primary producers levels, are not strong enough at other trophic levels to be detected by AMBI.

In fact, although a number of species composition shifts are already recognisable in qualitative terms, the benthic community structure in the three areas considered along the spatial gradient of eutrophication symptoms still exhibits, in a certain extent, a reasonably alike arrangement regarding the macrofaunal species (Marques *et al.*, 2003). In this case, AMBI values estimated in the Mondego estuary were similar at the three sampling areas due to the common dominance of *Hydrobia ulvae*, which belongs to ecological group III. Besides, all the other indicators were strongly affected by large abundances of *Hydrobia ulvae* and *Cerastoderma edule*, the dominant species. Nevertheless, such dominance does not have anything to do with pollution, being rather related to the availability of higher resources (Pardal *et al.*, 2000). Despite these difficulties, with regard to other impact sources (*e.g.* outfalls, oil platforms), AMBI revealed to be efficient in detecting stress gradients (Borja *et al.*, 2003a). In fact, the application of AMBI in the Escombreras basin and Cape Tiñoso case studies has lead to good results. For instance, in the case of Cape Tiñoso, even accounting only for Polychetes, AMBI was the only indicator able to differentiate, although not as clearly as that, the control stations closer to the floating cages' area. The good performance of AMBI is also evident in the Mira estuary, where all sampling stations are considered in good ecological status, which fully consistent with other authors (*e.g.* Costa *et al.*, 1994; Raposo *et al.*, 1996).

As a whole, results lead us to think that AMBI is a good tool to detect pollution. However, some precautions, already described by Borja *et al.* (2003a), have to be taken in order to observe a correct application. It is assumed that AMBI's robustness is reduced when only a very small number of taxa (1 to 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 & Zenetos (2002), 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 (G.I.) and opportunist species of first and second order.

Other indicators, like the Norwegian Indicator Species Index (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 the case of Norway, sensitive values for each species were determined after analysing 1080 samples from Norwegian fjords and coastal waters (1975 to 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 freely-available software, including a continuously updated species list (approximately 3000 taxa presently), which makes it especially convenient.

4.4.2. Indices based on ecological strategies

The Polychaetes/Amphipods Ratio was able to illustrate correctly the existence of an eutrophication gradient based on the Mondego estuary South arm intertidal communities. Nevertheless, in the other case studies, many samples did not allow to apply it simply due to the absence of amphipods. In such case, the ratio would reflect an extremely polluted scenario, which, we knew 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 Area and Betanzos (Atlantic Ocean), in our case studies it only worked well when applied to intertidal data. As, for instance, the Nematodes/Copepods Ratio used, which is used in 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 (Feeding Structure Index and Infaunal Trophic Index), our results have shown their inefficacy as reliable tools to detect 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 on the subtidal communities of the Mondego estuary, the ITI was allways inefficient in pointing up disturbance situations.

Also, in the Mar Menor or in the subtidal communities of the Mondego estuary, contrarily to what could be expected in accordance to Word (1990), ITI exhibited the highest values precisely in the less organically enriched areas. Actually, only in the case of the Mondego estuary intertidal communities ITI values showed significantly correlated with the amount of organic matter in the sediments. Currently, precisely in this case, the higher organic matter content in the sediment has a natural origin, the *Zostera noltii* meadows primary production. Therefore, ITI does not appear able to differentiate different situations along a gradient of eutrophication symptoms, which depend on the water column nutrients concentration and water circulation (Marques *et al.*, 2003).

Besides the bad results in the present case studies, there are other reasons to not recommend ITI use. One of the disadvantages of these indicators is the need for determining organisms' diet, which can only be achieved through the study of stomach's contents, by 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 these indicators were applied 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 (Fauchald & Jumars, 1979). Also, *Heteromastus filiformis*, which it is classified by Word (1990) as surface detrital feeder, is considered as subsurface deposit feeder by Brown *et al.* (1985). And what is more, while Word (1990) classifies most of the carnivore species in group 2 (surface detrital feeders), Codling & Ashley (1992) consider that they should belong to group 3 (surface deposit feeders), as most of them consume particles bigger than 50 micres in size. Another problem in determining the trophic category of many Polychaete species is their alternative feeding behaviour, which can occur under certain circumstances. For instance, from laboratory experiments, Buhr (1976) determined that the terebellid *Lanice conchylega*, considered as a detritivore, changes into a filter feeder when phytoplankton reaches a given concentration in the water column. Also, Taghon *et al.* (1980) observed that some species of the Spionidae family, usually taken for detritivores, could change into filterers, modifying the palps into a characteristic helicoidal shape. On the other hand, some species of the Sabellidae and Owenidae families can shift from filterers to detritivores. And we can consider that some omnivore and detritivore species changed into carnivores when they consume the rests of other animals (Dauer *et al.*, 1981; Maurer & Leathem, 1981). All these examples lead to doubts about the existence of a clear separation between different feeding strategies. That is why other characteristics, such as the degree of individual's mobility and the morphology of the mouth parts must be included in the definition of Polychaete trophic categories (Gambi & Giangrande, 1985). Different combinations of such characteristics constitute what Fauchald & Jaumars (1979) named «feeding guilds».

On the benthic systems studies, namely when identifying different types of impacts, authors like Maurer *et al.* (1981), Dauer (1984) and Pires & Múniz (1999) have tried, with good results, to classify the different polychaetes species in feeding guilds. The main problem in applying such a kind of classifications is the determination of the possible combinations for each species. Actually, according to Dauer (1984), many families hold more than one combination depending on the type of feeding they follow, their grade of mobility and the morphology of their mouth apparatus being, therefore, monospecific every combination. In practice, very often, such a classification does not have much sense.

Some of these questions, and the fact that the trophic groups classification in the case of ITI 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.

4.4.3. Biodiversity as reflected in diversity measures

Regarding diversity measures, the Margalef Index was the one showing the best performance, despite its relative simplicity as compared to other indices, namely the ones accounting for species richness and individuals abundance. Actually, it successfully differentiated distinct eutrophication levels in the Mondedo estuary South arm intertidal communities, and was also effective in detecting organic enrichment situations in the Mar Menor lagoon. As for the the Shannon-Wiener, Simpson and Berger-Parker indices, they appear to be too 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 Total Taxonomic Distinctness (TTD) was able to correctly distinguish between different scenarios along the gradient of eutrophication symptoms in the South arm of the Mondego estuary. Moreover, together with AMBI and the Margalef Index, TTD showed able to discriminate between more and less organically enriched areas in Mar Menor. In all our case studies, TTD appears significantly correlated to the Margalef Index, and in the case of the intertidal communities in the Mondego estuary it shows actually to be the most sensitive out of the two. Nonetheless, Warwick & Clarke (1998) consider not recommendable the use of that measure due to, in general, TTD 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 they cover many of the features (*e.g.* independency on simple size/effort or monotonic response to environmental degradation) required in order to be a good diversity indicator, in view of our results, the other measures proposed by Warwick & Clarke (1995; 1998) did not show any advantage as compared to other diversity indices. An exception was the Cape Tiñoso case study, where we observed a situation of intermediate organic enrichment, susceptible in some cases of favouring an increase in diversity. Here, contrarily to what happens with oher diversity indices, Average Taxonic Distinctness is not positively correlated with the concentration of chlorophyll *a* in the water column, exhibiting, in fact, a monotonic answer to stress.

The fact that, in general, Taxonomic Distinctness measures do not appear to be more sensitive to environmental stress, as compared to other diversity indices, has also been observed by Somerfield *et al.* (1997) in studies on the North Sea oil fields impact and Hall & Greenstreet (1998), studying fish communities, found that Taxonomic Distinctness measures showed identical trends to conventional diversity indices. Yet, Somerfield *et al.* (2003) have proposed Average Taxonomic Distinctness to be use as tool to classify the ecological status when implementing the European Water Framework Directive. This recommendation is due to an apparent advantage of this index, which is the fact that it includes a master list of *taxa* corresponding to what is assumed to represent reference conditions. Moreover, the software includes a statistical framework from which to measure the departure from what is expected (the reference condition).

In spite of such advantages, a study by Prior *et al.* (2004) suggests that before considering Taxonomic Distinctness measures as applicable in implementing the European Water Framework Directive, some modifications have to be introduced. In fact, investigations have shown that Taxonomic Distinctness is sensitive to the frequency of occurrence of taxa across each sample. This is contrary to the null hypothesis upon which Average Taxonomic Distinctness is currently based, that takes into account the natural spatial variation caused by reproductive strategies within benthic communities. That is why Prior *et al.* 2004 underline the need that frequency distribution is well studied for high ecological status, in order to set a strong reference from which to measure departures.

It is interesting to observe how the two tested indices based on specific richness (Margalef index and Total Taxonomic Distinctness) were the most successful measures in differentiating the diverse grades of pollution, leading us to think that the increment or decrement in the number of species is one of the best disturbance indicators, and therefore, essential when it comes to differentiating ecological status.

The Northeast Atlantic Geographical Intercalibration Group Benthic Expert (NEAGIG, 2004) considered that the selected metrics to be used in the European Water Framework Directive context need to distinguish clearly across the good/moderate boundary. Obviously, those two measurements are not able themselves alone to work out such distinction, as they will always need a previous knowledge on the number of species (reference situation) of the studied site. In that sense, few are the indices capable of establishing the different ecological status (high, good, moderate, poor and bad).

The other inconvenience of species richness is that, contrarily to Taxonomic Distinctness, it 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 (Warwick & Clarke, 1998; Leonard *et al.*, in press). Indeed, the fact that the Average Taxonomic Distinctness sustained high values in Mondego stations with few species but low levels of organic matter and therefore not organically polluted, showed the ability of this index to detect impacts despite possible natural environmental disturbances, as for example salinity fluctuations, in an estuary or coastal lagoon. Those salinity fluctuations in the Mondego estuary do not seem to be the determining factor that influenced the species richness. The previous mentioned measure can be affected by a number of factors, as for example the marine water and freshwater inputs, that make difficult the colonisation and settlement possibilities of certain species. Nevertheless, in the Mar Menor the salinity was a very influential parameter because it is correlated with the confinement or isolation degree in the lagoon (Gamito *et al.,* 2005; Pérez-Ruzafa & Marcos, 1992; Perez Ruzafa *et al.,* 2005)

Studies like Heino *et al.* (2005) showed that Taxonomic Distinctness also varies along natural gradients and it is unlikely that a site can be determined to be degraded or not degraded based only on this measure. On the other hand, although Average Taxonomic Distinctness has the ability to discriminate properly between polluted and non polluted areas in those with low number of species (as it is the case of the subtidal communities in Mondego), the results of this study demonstrated that its power of discrimination decreases when the species number increases (see confidence limits in the funnel graphic representation, Figure 10), which leads us to think that the index is not able to show correlations with pollution in areas where richness depends on other factors.

4.4.4. Indicators based on species biomass and abundance

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

Nevertheless, the dominance of few species with small-size individuals, although characteristic of polluted environments, may occur in non-polluted environments, which is not unusual (the Mondego estuarine benthic community constitutes a good example), which may lead to erroneous ecological status assessments. This problem has in fact been perceived in several case studies (Ibanez & Dauvin, 1988; Beukema, 1988; Weston, 1990; Craeymeersch, 1991), and is the reason why the W-Statistic was not very successful in detecting organic pollution in the Mar Menor lagoon or at the Escombreras basin. A possible explanation is the fact that the W-Statistic was wholly developed to assess the impact of organic pollution, and in these two study areas, although sediment organic enrichment is a concern, there are also other kinds of pollution (*e.g.* heavy metals), and different types of environmental stress.

4.4.5. 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 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 the case of the Mar Menor marine lagoon, despite responding to sediment organic enrichment, both the Exergy Index and Specific Exergy were unable to distinguish between areas affected by organic pollution and areas that are not. Nevertheless, the Exergy Index worked pretty well regarding the Mondego estuary intertidal communities, being able to distinguish between different areas along a gradient of eutrophication symptoms. These differences in efficiency might be due to the fact that in the Mar Menor lagoon the effects of organic pollution are to a certain extent covered up by other system structuring factors, while in the South arm of the Mondego estuary eutrophication is undoubtedly the major driving force behind the ongoing changes.

Finally, 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 weighting factors expressing the quality of biomass play a major role in estimations.

4.4.6. 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 pretty 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*. (1999) considered four metrics in order to use B-IBI in Carolina: a) mean abundance, b) mean number of taxa, c) 100 minus percent abundance of the top two numerical dominants, and d) percent abundance of pollution sensitive taxa, 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, than the one proposed by Weisberg (1997).

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, and such constraints lead us to discourage the generalised application of B-IBI.

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CHAPTER 5

COMBINING INDICATORS TO CHARACTERISE A SYSTEM'S ECOLOGICAL QUALITY STATUS

Ecological indicators are used in monitoring, assessment, and management of natural resources. As a result, the natural complexity of ecological systems represents a difficulty when selecting appropriate indicators to deal with such questions. Therefore, it is usually necessary to use a suite of indicators representative of the structure, function and composition of these ecological systems.

Our results demonstrate that none of the available measures of disturbance effects may be considered ideal. Thus, in our opinion, we should always considerer a correct combination of a number of indicators in order to make up for the shortcomings of each one of them, which can result in a good toolset for determining system's ecological quality status. Let us exemplify this in more detail.

According to our results, for instance, AMBI, Margalef Index and Total Taxonomic Distinctness were the most sensitive indicators in discriminating disturbance situations in our case studies. They must therefore be considered as powerful tools in any multimetric approach to establish ecological levels. Nevertheless, while AMBI does not require reference values, the Margalef index and Total Taxonomic Distinctness do so. This makes difficult their integration in a combined approach, because it will be necessary to establish a correspondence between indicators' values and ecological status.

Although Total Taxonomic Distinctness has showed to be more sensitive than the Margalef Index in our study on the intertidal communities of the Mondego estuary, it is preferable not to integrate it in methodologies involving the combination of different indicators, since according to Clarke & Warwick (1999), it is of restricted applicability. Actually, these authors consider that this indicator tends to track species richness rather closely and will only be 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.

On the other hand, we think that the Shannon-Wiener Index, although it did not work as well as the Margalef one, has to be taken into account, since diversity measures must address other aspects (*i.e.* proportional abundance of individuals), apart from species richness. Moreover, although it requires reference values, the fact that the Shannon-Wiener Index is probably the most used diversity measure in environmental studies makes relatively easy to establish correspondences between its values and different ecological levels.

Despite the above mentioned difficulties, the complementary use of different indicators or methods, based on different ecological principles, is highly recommendable in determining the environmental quality status of an ecosystem.

Interestingly, the European Water Framework Directive, WFD, (EC, 2000) implementation, for instance, appears as a good field of application. Indeed, since this Directive became effective, the approach to water issues has changed significantly. Its main goal is to achieve good ecological status in transitional and coastal waters, until 2015.

The Ecological Status of a water body is determined using a range of hydromorphological and physico-chemical quality elements as well considering biological quality elements. In the case of transitional waters, the biological quality elements include phytoplankton, other aquatic flora, benthic invertebrate fauna and fish fauna. In the case of coastal waters, the biological elements include phytoplankton, other aquatic flora, benthic invertebrate fauna but not fish fauna.

Accordingly, in the scope of this Directive, macrobenthos is one of the biological quality elements to consider for both transitional and coastal waters. Beside the central functioning role that benthic macrofauna has in marine/estuarine ecosystems, various studies have also frequently demonstrated that it responds relatively rapid to anthropogenic and natural stress (Pearson & Rosenberg, 1978; Dauer, 1993). Due to their limited mobility, benthic communities are quite sensitive to local disturbance, and due to their permanence over seasonal time scales, they integrate the recent history of disturbances which might not be detected in the water column (Bettencourt *et al.*, 2004). In benthic communities, we can also find different species exhibiting different tolerances to stress (Dauer, 1993), which covers the WFD demand of integrating sensitive species.

After the TICOR Project guidelines (Bettencourt *et al.*, 2004), the following metrics have been proposed to assess ecological quality regarding macrobenthos element: a) **abundance**: the metrics selected for this were the Shannon-Wiener and Margalef indices, since these indices provide complementary diversity measures. Shannon-Wiener index takes proportional abundance of species into account, while Margalef index focus on species richness and b) **composition**: the metric selected for composition of macrobenthos was the AMBI, once it is based on the presence of sensitive species and pollution indicator species.

After the calculation of ecological indices, a multimetric methodology is here anticipated to assess the Ecological Quality Ratio (EQR) and the Ecological Quality Status (EQS) of coastal and transitional waters systems. This methodology was initially proposed by Bald *et al.* (2005) to assess the physico-chemical EQS, also within the WFD scope, being afterwards adapted by Muxika *et al.* (submitted) to the macrobenthos quality assessment.

Indices results are combined in a factor analysis (FA) according to Bald *et al.* (2005). The extraction method of the factor analysis principal components (PCA) is applied to the results of each station, in each sampling period, and virtual reference stations for High and Bad ecological Status are also considered in the analysis. The FA, through the method of the principal components extraction, allows the study of interrelations between a large number of variables, explaining them in terms of their underlying dimensions (factors). Since the extreme reference conditions are accounted for, it is possible to define, in the three-dimensional space, the real stations' position relatively to the virtual reference stations.

Data are previously normalised, Log (1+X) transformed, and standardised, subtracting the mean and dividing by the standard deviation. In the analysis the Varimax rotation method is adopted to make results interpretation easier. From this analysis the scores of the first 3 factors are extracted.

After obtaining the sampling stations' relative position (scores extracted from the FA), the projection of each sampling station in the axis connecting both reference stations (high and bad status) is calculated in the new three--dimensional space created by the FA (Figure 15).

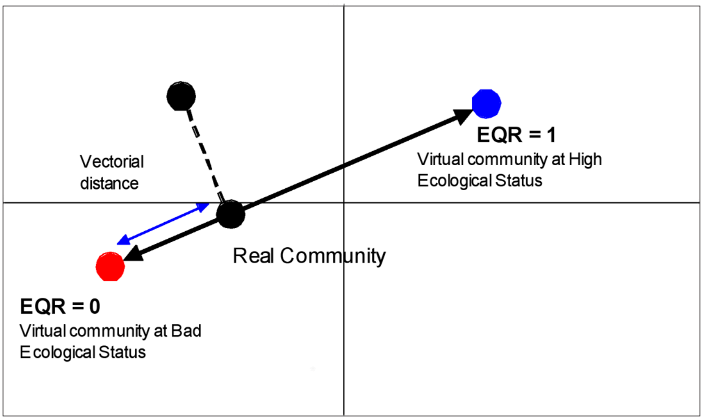

Figure 15. Principal Components Analysis showing virtual communities at high and bad ecological status, and relative position of real community (after Borja *et al.*, 2003).

Subsequently, the Euclidean Distance of each projection to the virtual station possessing bad status is measured. The value of 1 (accordingly to the definition of EQR in the WFD) is attributed to the distance between both virtual reference stations (Bad and High). So stations in better condition, with higher Ecological Status, will achieve values near 1, while stations in worse ecological condition will be located nearer bad reference station and will assume values nearer 0. The boundaries along this EQR axis (from 0 to 1) should be defined, reflecting each of the 5 ecological classes according to WFD normative definitions.

Despite WFD indications for reference conditions to be type-specific, in some situations, in order to proceed to a correct ecological evaluation of the systems, the same reference conditions might not be adequate to the entire system. In the case of transitional waters such as estuaries, since there is a strong salinity gradient from the mouth to the head, a slightly different situation should be considered. For effectiveness of the proposed methodology, it is necessary to define stretches within these systems where specific reference conditions should rule. This is to ensure that the natural biological impoverishment that is observed along the salinity gradient towards the inner parts of transitional waters systems is reflected and accounted for by the ecological evaluation of multimetric methodologies. Other aspects such as morphological, hydrological or habitat type, might also help to the definition of specific stretches among transitional systems in evaluation (Ferreira *et al.*, 2006).

Although we are still in a developing phase, it is our strong believe that the right use of such a multimetric approach can be of great utility in establishing ecological quality status in environmental studies. Hopefully, in a near future, empirical studies will provide us examples of the performance of such approach.

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REFERENCES

Abou-Aisha, K.H., Kobbia, I.A. Abyad, M.S., Shabana, E.F. & Schanz, F. 1995. Impact of phosphorous loadings on macro-algal communities in the Red sea coast of Egypt. *Wat. Air, Soil Pollut.,* 83: 285-297.

Agard, J.B., Gobin, J. & Warwick, R.M. 1993. Analysis of marine macrobenthic community structure in relation to pollution, natural oil seepage and seasonal disturbance in a tropical environment (Trinidad, West Indies). *Mar. Ecol. Prog. Ser.*, 92: 233-243.

Alba, T.J. & Sánchez, O.A. 1988. Un método rápido y simples para evaluar la calidad biológica de las águas correntes basado en el de Hellawell (1978). *Limnética*, 4:51-56.

Albrecht, W.N., Woodhouse, C.A. & Miller, J.N. 1981. Nearshore dredge-spoil dumping and cadmium, copper and zinc levels in a dermestid shrimp. *Bull. Environ. Contamin. Toxicol.*, 26: 219-223.

Anderlini, V.C. & Wear, R.G. 1992. The effects of sewage and natural seasonal disturbance on benthic macrofaunal communites in Fitzray Bay, Wellington, New Zealand. *Mar. Pollut. Bull.*, 24: 21-26.

Andersen, A.N. 1997. Using ants as bioindicators: multiscale issues in ant community ecology. Conser. Ecol. (online). http://consecol.org/vol1/iss1/atr8.

Andrade, F. 1986. O estuário do Mira: caracterização geral e análise quantitativa da estrutura dos macropovoamentos bentónicos. PhD Thesis, University of Lisbon, Portugal.

Anger, K. 1975. On the influence of sewage pollution on inshore benthic communities in the south of Kiel Bay. 2. Quantitative studies on community structure. *Helgoländer. Wiss. Meeresunters*, 27: 408-438.

Bald, J., Borja, A., Muxika, I., Franco, J. & Valencia, V. 2005. Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: A case-study from the Basque Country (Northern Spain). *Marine Pollution Bulletin*, 50: 1508-1522.

Bazzaz, F.A. 1986. Life history of colonizing plants: some demographic, genetic, and physiological features. In: Mooney, H.A. & Drake, J.A. (Eds.), Ecology *of Biological Invasions of North America and Hawaii*. Springer-Verlag, Berlin, 96–110 pp.

Bellan, G. 1980. Annélides polychétes des substrats solids de troits mileux pollués sur les côrtes de Provence (France): Cortiou, Golfe de Fos, Vieux Port de Marseille. *Téthys*, 9(3): 260-278.

Bellan-Santini, D. 1980. Relationship between populations of amphipods and pollution. *Mar. Pollut. Bull.*, 11: 224-227.

Belsher, T. & Boudouresque, C.F. 1976. L'impact de la pollution sur la fraction algale des peuplements benthiques de Méditerranée. *Atti Tavola Rotonda Internazionale*, *Livorno*: 215-260.

Belsher, T. 1982. Measuring the standing crop of intertidal seaweeds by remote sensing. In: Last, F.T., Hotz, M.C. & Bell, B. (Eds.), *Land and its Uses Actual and Potential: An Environmental Appraisal.* NATO Seminar on Land and its Uses, Edinburgh 19 Sep.-1 Oct. 1982, 10: 453- 456.

Bettencourt, A., Bricker, S.B., Ferreira, J.G., Franco, A., Marques, J.C., Melo, J.J., Nobre, A., Ramos, L., Reis, C.S., Salas, F., Silva, M.C., Simas, T & Wolff, W. 2004. *Typology and reference conditions for Portuguese transitional and coastal waters*. INAG. IMAR. 99 p.

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

Blair, R.B. 1996. Land use and avian species diversity along an urban gradient. *Ecol. Applic.*, 6: 506-519.

Bonne, W., Rekecki, A. & Vincx, M. 2003. Impact assessment of sand extraction on subtidal sandbanks using macrobenthos. In: *Benthic copepod communities in relation to natural and anthopogenic influences in the North Sea*. Ph.D Thesis of W. Bonne, Ghent University, Biology Department, Marine Biology Section, Belgium, 207-226 pp.

Borja, Á., Franco, J. & Pérez, V. 2000. A Marine Biotic Index to Establish the Ecological Quality of Soft-Bottom Benthos Within European Estuarine and Coastal Environments. *Mar. Pollut. Bull.*, 40(12): 1100-1114.

Borja, Á., García de Bikuña, B., Blanco, J.M., Agirre, A., Aierbe, E., Bald, J., Belzunce, M.J., Fraile, H., Franco, J., Gandarias, O., Goikoetxea, I., Leonardo, J.M., Lonbide, L., Moso, M., Muxika, I., Pérez, V., Santoro, F., Solaun, O., Tello, E.M. & Valencia, V. 2003a. *Red de Vigilancia de las masas de agua superficial de la Comunidad Autónoma del País Vasco. Tomo 1: Metodologías utilizadas*. Departamento de Ordenación del Territorio y Medio Ambiente, Gobierno Vasco, 199 p.

Borja, Á., Muxika, I. & Franco, J. 2003b. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. *Mar. Pollut. Bull.*, 46: 835-845.

Borja, Á., Franco, J. & Muxika, I. 2003c. Classification tools for marine ecological quality assessment: the usefulness of macrobenthic communities in an area affected by a submarine outfall. *ICES CM 2003/Session J-02*, Tallinn (Estonia), 24-28 September, 2003.

Bratton, S.P., Hapeman, J.R. & Mast, A.R. 1994. The Lower Susquehanna River Gorge and floodplain (U.S.A.) as a riparian refugium for vernal, forest-floor herbs. *Cons. Biol.*, 8: 1069-1077.

Brock, V. 1992. Effects of mercury on the biosynthesis of porphyrins in bivalve molluscs (*Cerastoderma edule* and *C .lamarcki*). *J. Exp. Mar. Bio. Ecol.*, 164: 17-29.

Browder, S.F., Johnson, D.H. & Ball, I.J. 2002. Assemblages of breeding birds as indicators of grassland condition. *Ecol. Indic.*, 2: 257-270.

Brown, A.C. 1985. The effects of crude oil pollution on marine organisms: a literature review in the South African context: conclusions and recommendations. *S. Afr. Natl. Scient. Progr. Rep.* No. 99, 33 p.

Bryan, G.W. & Gibbs, P.E. 1987. Polychaetes as indicators of heavy-metal availability in marine deposits. In: Capuzzo, J.M. & Kester, D.R. (Eds.), *Oceanic processes in marine pollution, vol 1, Biological processes and Wastes in the Ocean*. Krieger Publishing Co. Inc., Melbourne, 37-49 pp.

Buhr, K.J. 1976. Suspension feeding and assimilation efficency in *Lanice conchilega* (Polychaeta). *Mar. Biol.*, 38: 373- 383.

Calderón-Aguilera, L.E. 1992. Análisis de la infauna béntica de Bahía de San Quintín, Baja California, con énfasis en su utilidad en la evaluación de impacto ambiental. *Cienc. Mar.*, 18(4): 27-46.

Canterbury, G.E., Martin, T.E., Petit, D.R., Petit, L.J. & Bradford, D.F. 2000. Bird communities and habitat as ecological indicators of forest condition in regional monitoring. *Conserv. Biol.*, 14: 544–558.

Carell, B., Forberg, S., Grundelius, E., Henrikson, L., Johnels, A., Lindh, U., Mutvei, H., Olsson, M., Svaerdstroem, K. & Westermark, T. 1987. Can mussel shells reveal environmental history? *Ambio*, 16(1): 2-10.

CARM. 1998. Ecosistemas de nuestra región. El medio marino. Agencia para el Medio Ambiente y la Naturaleza. Murcia.

Carmichael, J., Richardson, B., Roberts, M & Jordan. S.J. 1992. *Fish sampling in eight Chesapeake Bay tributaries*. Maryland Dept. of Natural Resources, CBRM-HI-92-2. Annapolis, 50 p.

Carpene, E. 1993. Adaptations of marine organisms to heavy metal pollution: Metallothioneins as biological markers. *Ital. J. Biochem.*, 42(5): 306.

Casselli, C., Ponti, M. & Abbiati, M. 2003. Valutazione della qualità ambientale della laguna costiera Pialassa Baiona attraverso lo studio dei suoi popolamenti bentonici. *XIII Congresso Societá Italiana de Ecología,* Como, Villa Olmo, 8-10 September 2003, poster.

Chutter, F.M. 1972. An empirical biotic index of the quality of water in South African streams and rivers. *Water Res.*, 6: 19-30.

Clarke, K.R. 1990. Comparison of dominance curves. *J. Exp. Mar.Biol. Ecol.*, 138(1-2): 130-143.

Clarke, K.R & Warwick, R.M. 1999. The taxonomic distinctness measures of biodiversity: weighting of steps lengths between hierarchical levels. *Mar. Ecol. Prog. Ser.*, 184: 21-29.

Clarke, K.R. & Warwick, R.M. 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Mar. Ecol. Prog. Ser.*, 216: 265-278.

Codling, I.D. & Ashley, S.J. 1992. Development of a biotic index for the assessment of pollution status of marine benthic communities. Final report to SNIFFER and NRA. NR 3102/1.

Cooper, J.A., Harrison, T.D., Ramm, A.E. & Singh, R.A. 1993. *Refinement,Enhancement and Application of the Estuarine Health Index to Natal'sEstuaries, Tugela - Mtamvuna*. Unpublished Technical Report. CSIR, Durban.

Cooper, R.J., Langlois, D. & Olley, J. 1982. Heavy metals in Tasmania shell fish. I. Monitoring heavy metals contamination in the Dermwert Estuary: use of oysters and mussels. *Jat*, 2(2): 99-109.

Cormaci, M. & Furnari, G. 1991. Phytobenthic communities as monitor of the environmental conditions of the Brindisi coast-line. *Oebalia,* XVII-I suppl.: 177-198.

Cossa, A. & Rondeau, J.G. 1985. Seasonal geographical and size -induced variability in mercury content of *Mytilus edulis* in an estuarine environment: a re-assessment of mercury pollution level in the Estuary and Gulf of St.Lawrence. *Mar. Biol.,* 88: 43-49.

Cossa, F., Picard, M. & Gouygou, J.P., 1983. Polynuclear aromatic hydrocarbons in mussels from the Estuary and northwestern Gulf of St. Lawrence, Canada. *Bull. Environ. Contam. Toxicol.*, 31: 41-47.

Costa, M.J., Almeida, P.R., Costa, J.L. & Assis, C.A. 1994. Do eel grass beds and salt marsh borders act as preferential nurseries and spawning grounds for fish? – An example of the Mira estuary in Portugal. *Ecological Engineering,* 3: 187–195.

Couch, J.A. & Harshbarger, J.C. 1985. Effects of carcinogenic agents on aquatic animals: An environmental and experimental overview. *Environ. Carcigneosis Revs.*, 3C(1): 63-105.

Craeymeersch, J.A. 1991. Applicability of the abundance/biomass comparison method to detect pollution effects on intertidal macrobenthic communities. *Hydrobiol. Bull.*, 24(2): 133-140.

Dabbas, M., Hubbard, F. & Mc Manus, J. 1984. The shell of *Mytilus* as an indicator of zonal variatons of water quality within an estuary. *Estuar. Coast. Shelf. Sci.,* 18(3): 263-270.

Dale, V.H. & Beyeler, S.C. 2001. Challenges in the development and use of ecological indicators. *Ecol. Indic.*, 1: 3-10.

Dale, V.H., Beyeler, S.C. & Jackson, B. 2002. Understorey vegetation indicators of anthropogenic disturbance in longleaf pine forests at Fort Benning, Georgia, USA. *Ecol. Indic.*, 1: 155–170.

Dauer, D.M. 1984. The use of polychaete feeding guilds as biological variables. *Mar. Pollut. Bull.*, 15 (8): 301-304.

Dauer, D.M., Maybury, C.A. & Ewing, R.M. 1981. Feeding behavior and general ecology of several spionid solychaetes from the Chesapeake Bay. *J. Exp. Mar. Biol. Ecol.*, 54 (1): 21-38.

Dauer, D.M., Luckenbach, M.W. & Rodi, A.J., 1993. Abundance-biomass comparison ABC method: effects of an estuarine gradient, anoxic/hypoxic events and contaminated sediments. *Mar. Biol.*, 116: 507-518.

De Boer, W.F., Daniels, P & Essink, K. 2001. Towards ecological quality objectives for North Sea benthic Communities. National Institute for Coastal and Marine Management (RIKZ), Haren, the Netherlands. Contract RKZ 808, Report nr 2001-11, 64 p.

De Paw, N. & Vanhooren, G. 1983. Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*, 100: 153-168.

de Ruiter P.C., Neutel, A.M. & Moore, J.C. 1994. Food webs and nutrient cycling in agro-ecosystems *Trends in Ecology and Evolution*, 9/10, 378-383.

de Ruiter P.C., Neutel, A.M. & Moore, J.C. 1995. Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science*, 269, 1257-1260.

De Wolf, P. 1975. Mercury content of mussel from West European Coasts. *Mar. Pollut. Bull.*, 6 (4): 61-63.

Deegan, L.A., Finn, J.T., Ayvazian, S.G. & Ryder, C. 1993. *Feasibility and application of the Index of Biotic Integrity to Massachusetts Estuaries (EBI)*. Final report to Massachusetts Executive Office of Environmental Affairs. North Grafton, MA: Department of Environmental Protection.

DeKeyser , E.S., Kirby, D.R. & Ell, M.J. 2003. An index of plant community integrity: development of the methodology for assessing prairie wetland plant communities. *Ecol. Indic.*, 3: 119-133.

Delong, M.D. & Brusven, M.A. 1998. Macroinvertebrate community structure along the longitudinal gradient of an agriculturally impacted stream. *Environ. Manage.,* 22: 445-457.

Docampo, L. & Bikuña, B. 1991. Analysis of the physico-chemical variables of the stream waters of Vizcaya (Basque Country): 1. Mathematical model of conductivity. *Arch. Hydrobiol.*, 122 (3): 351-372.

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

Drayton, B. & Primack, R.B. 1996. Plant species lost in an isolated conservation area in metropolitan Boston from 1894 to 1993. *Conserv.* Biol., 10: 30-39.

Dzwonko, Z. & Loster, S. 1992. Species richness and seed dispersal to secondary woods in southern Poland. *J. Biogeog.*, 19: 195-204.

Eadie, J.B., Faust, W., Gardner, W.S. & Nalepa, T. 1982. Polycyclic aromatic hydrocarbons in sediments and associated benthos in Lake Erie. *Chemosphere*, 11: 185-191.

EC, 2000. Directive of the European Parliament and of the Council 2000/60/EC establishing a framework for community action in the field of water policy PE-CONS 3639/1/00, 72 p.

Encalada, R.R. & Millan, E. 1990. Impacto de las aguas residuales industriales y domesticas sobre las comunidades bentónicas de la Bahía de Todos Santos, Baja California, Mexico. *Cinc. Mar.*, 16(4): 121-139.

Engle, V., Summers, J.K. & Gaston, G.R. 1994. A benthic index of environmental condition of Gulf of Mexico. *Estuaries*, 17(2): 372-384.

EPA. 1987. Biological criteria for the protection of the aquatic life. Vols I-III. Surface Water Section, Division of Water Quality Monitoring and Assessment, Ohio Environmental Protection Agency, Columbus, OH.

Fano, E.A., Mistri, M. & Rossi, R. 2003. The ecofunctional quality index (EQI): a new tool for assessing lagoonal ecosystem impairment. *Estuar. Coast. Shelf Sci.*, 56: 709-716.

Fasham, M.J.R. 1984. Flows of energy and materials in marine ecosystems: theory and practice. NATO Conf Ser IV. Marine science. Plenum, New York, 733 p.

Fauchald, K. & Jumars, P. 1979. The diet of the worms: a study of Polychaete feeding guilds. *Oceanogr. Mar. Biol. Ann. Rev.*, 17: 193-284.

Ferreira, J.G., Nobre, A.M., Simas, T.C., Silva, M.C., Newton, A., Bricker, S.B., Wolff, W.J., Stacey P.E. & Sequeira, A. 2006. A methodology for defining homogeneous water bodies in estuaries - Application to the transitional systems of the EU Water Framework Directive. *Estuarine, Coastal and Shelf Science,* 66: 468-482.

Finn, J.T. 1976 Measures of ecosystem structure and function derived from analyses of flows. *Journal of Theoretical Biology*, 41: 535-546.

Fisher, R.A., Corbet, A.S. & Williams, C.B. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. *J. Anim. Ecol.*, 12: 42-58.

Fonseca, J.C., Marques, J.C., Paiva, A.A., Freitas, A.M., Madeira, V.M.C. & Jørgensen, S.E. 2000. Nuclear DNA in the determination of weighing factors to estimate exergy from organisms biomass. *Ecol. Model.,* 126: 179-189.

Fonseca, J.C., Pardal, M.A., Azeiteiro, U.M. & Marques, J.C. 2002. Estimation of ecological exergy using weighing factors determined from DNA contents of organisms – a case study. *Hydrobiologia*, 475/476: 79-90

Forni, G. & Occhipinti Ambrogi, A. 2003. Applicazione del coefficiente biotico (Borja *et al.,* 2000) alla comunità macrobentonica del Nord Adriático. *Meeting of the Italian Society of Marine Biology*, Tunisia, June 2003.

Frontier, S. & Pichod-Viale, D. 1995. Ecosystèmes: Structure, Fonctionnement, Évolution, 2nd edn. Masson, Paris, 447 p.

Fuliu, X. 1997. Exergy and estructural exergy as ecological indicators for the state of the Lake Chalou ecosystem. *Ecol. Model.*, 99: 41-49.

Fuller, R.J., Gough, S.J. & Marchant, J.H. 1995. Bird populations in new Lowland woods: landscape, design and management perspectives. In: Ferris-Kaan, R (Ed.), *The ecology of woodland creation*. London:John Wiley & Sons Ltd, 163–182 pp.

Gambi, M.C. & Giangrande, A. 1985. Characterization and distribution of polychaete trophic groups in the soft-bottoms of the Gulf of Salerno. *Oebalia*, 11(1): 223-240.

Gamito, S., Gilabert, J., Marcos, C. & Pérez-Ruzafa, A. 2005. Effects of changing environmental conditions on lagoon ecology. In: Gönenc, J.E. & Wolflin, J. (Eds.), *Coastal lagoons: ecosystem processes and modelling for sustainable use and development*. CRC Press, Boca Raton, 193-229 pp.

Gauch, H.G. 1982. *Multivariate analysis in community ecology. Cambridge Studies in Ecology*. Cambridge University Press. New York.

Gee, J.M., Warwick, R.M., Schaanning, M., Berge, J.A. & Ambrose, W.G. 1985. Effects of organic enrichment on meiofaunal abundance and community structure in sublittoral soft sediments. *J. Exp. Mar. Biol. Ecol.*, 91(3): 247-262.

Gibbs, P.E., Langston, W.J., Burt, G.R. & Pascoe, P.L. 1983. *Tharyx marioni* (Polychaeta): A remarkable accumulator of arsenic. *J. Mar. Biol. Assoc. U.K.*, 63(2): 313-325.

Glemarec, M. & Hily, C. 1981. Perturbations apportées a la macrofaune benthique de la Baie de Concarneau par les effluents urbains et portuaries. *Acta Oecol. Appl.*, 2(2): 139-150.

Goff, F.G & Cottam, G. 1967. Gradient analysis: The use of species and symthetic indices. *Ecology*, 48:793-806.

Goldberg, E., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, P.L., Parker, P.L., Risebrough, R.W., Robertson, W., Schnider, E. & Gamble, E. 1978. The Mussel Watch. *Environ. Conserv.*, 5: 101-125.

Golovenko, V.K., Shchepinsky, A.A. & Shevchenko, V.A. 1981. Accumulation of DDT and its metabolism in Black Sea Mussels. *Izv. Akad. Nauk. SSR. Ser. Biol.,* 4: 453-550.

Gómez-Gesteira, J.L. & Dauvin, J.C. 2000. Amphipods are good bioindicators of the impact of oil spillson soft bottom macrobenthic communities. *Mar. Pollut. Bull.*, 40(11):1017-1027.

Gorostiaga, J.M., Borja, Á., Díez, I., Francés, G., Pagola-Carte, S. & Sáiz-Salinas, J.I. 2004. Recovery of benthic communities in polluted systems. In: Borja, Á. & Collins, M. (Eds.), *Oceanography and Marine Environment of the Basque Country, Elsevier Oceanography Series*, Elsevier, Amsterdam, 70: 549-578.

Gosset, R.W., Brown, D.A. & Young, D.R. 1983. Predicting the bioaccumulation of organic compounds in marine organisms using octanol/water partition coefficients. *Mar. Pollut. Bull.*, 14: 387-392.

Gray, J.S. 1979. Pollution-induced changes in populations. *Phil. Trans. R. Soc. London*, 286: 545-561.

Gray, J.S. & Mirza, F.B. 1979. A possible method for the detection of pollution induced disturbance on marine benthic communities. *Mar. Pollut. Bull.*, 10: 142-146.

Gray, J.S., Aschan, M., Carr, M.R., Clarke, K.R., Green, R.H., Pearson, T.H., Rosenberg, R., Warwick, R.M. & Bayne, B.L. 1988. Analysis of community attributes of the benthic macrofauna of Frierfjord/Langesundfjord and in a mesocosm experiment. En: Warwick, R.M. & Clarke, K.R. (Eds.), *Biological effects of pollutants. Results of a practical workshop*, 46(1-3): 151-165.

Hall, S.J. & Greenstreet, S.P. 1998. Taxonomic distinctness and diversity measures: responses in marine fish communities. *Mar. Ecol. Prog. Ser.,* 166: 227-229.

Hammond, P.C. & Miller, J.C. 1998. Comparison of the biodiversity of Lepidoptera within three forested ecosystems. *Ann. Entomol. Soc. Am.*, 91: 323-328.

Hansen, P.D. & Addison, R.F. 1990. The use of mixed function oxidases (MFO) to support biological effects monitoring in the sea. *Copenhagen Denmark Ices.*, 1: 1- 9.

Harrison, F.L., Lam, J.R. & Novacek, J. 1988. Partitioning of metals among metal-binding proteins in the bay mussel, *Mytilus edulis. Response of marine organisms to pollutants,* 24(1-4): 167-170.

Harrison, T.D., Cooper, J.A., Ramm, A. E. & Singh, R.A. 1994. *Application of the Estuarine Health Index to South Africa's estuaries, Orange River – Buffels (Oos).* Unpublished Technical Report. CSIR, Durban.

Harrison, T.D., Cooper, J.A., Ramm, A.E. & Singh, R.A. 1995. *Application of the Estuarine Health Index to South Africa's estuaries, Palmiet - Sout.* Unpublished Technical Report. CSIR, Durban.

Harrison, T.D., Cooper, J.A., Ramm, A.E. & Singh, R.A. 1997. *Application of the Estuarine Health Index to South Africa's estuaries, Old Woman's – Great Kei.* Unpublished Technical Report. CSIR, Durban.

Harrison, T.D., Cooper, J.A., Ramm, A. E. & Singh, R.A. 1999. *Application of the Estuarine Health Index to South Africa's estuaries, Transkei*. Unpublished Technical Report. CSIR, Durba.

Heino, J., Soininen, J., Lappalainen, J. & Virtanen, R. 2005. The relationship between species richness and taxonomic distinctness in freshwater organisms. *Limnol. Oceanogr.* 50(3), 978-986.

Hellawell, V.M. 1986. *Biological monitors of freshwater pollution and environmental management.* Elsevier. London.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. *Great Lakes Entomol.*, 20: 31-19.

Hobbs, R.J. 1997. Can we use plant functional types to describe and predict responses to environmental change? In: Smith, T.M., Shugart, H.H. & Woodward, F.I. (Eds.), *Plant Functional Types their Relevance to Ecosystem Properties and Global Change*. Cambridge, University Press, Cambridge, 66–90 pp.

Holl, K.D. 1996. The effect of coal surface mine reclamation on diurnal lepidopteran conservation. *J. Appl. Ecol.,* 33: 225-236.

Holliday, N.J. 1991. Species responses of carabid berttles (Colepoptera: Carabidae) during post--fire regeneration of boreal forest. *Can. Entomol.,* 123: 1369-1389.

Hong, J. 1983. Impact of the pollution on the benthic community. *Bull. Korean Fish. Soc.*,16: 273-290.

Hughes, R.G. 1984. A model of the structure and dynamics of benthic marine invertebrate communities. *Mar. Ecol. Prog. Ser.*, 15(1-2): 1-11.

Hulbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. *Ecology,* 52: 577-586.

Ibanez, F. & Dauvin, J.C. 1988. Long-term changes (1977-1987) in a muddy fine sand *Abra alba--Melina palmata* community from the Western Channel: multivariate time series analysis. *Mar. Ecol. Prog. Ser.*, 19: 65-81.

ISO. 1984. Water assessment of the water and habitat quality of rivers by a macroinvertebrate "score". ISO/TC 147/SC 5/ WG 6 N 4, Draft proposal ISO/ DP 8689.

Jensen, K., Randlov, A. & Riisgard, H.U. 1981. Heavy metal pollution from a point source demonstrated by means of mussels, Mytilus edulis. *Chemosphere*, 10(7): 761-765.

Jordan, S.J., Vaas, P.A & Uphoff, J. 1990. Fish assemblages as indicators of environmental quality in Chesapeake Bay. In: *Biological Criteria: Research and Regulation*, December 12-13, 1990, Arlington, VA. U.S. Environmental Protection Agency, Office of Water.

Jørgensen, S.E. 1994. Review and comparison of goal functions in system ecology. *Vie Milieu*, 44(1):11-20.

Jørgensen, S.E. & Mejer, H. 1979. A holistic approach to ecological modelling. *Ecol. Model.*, 7:169-189.

Jørgensen, S.E. & Mejer, H. 1981 Exergy as a key function in ecological models. In: Mitsch, W., Bosserman, R.W. & Klopatek, J.M. (Eds.), *Energy and ecological modelling. Developments in environmental modelling*, Elsevier. Amsterdam, 1: 587-590.

Jørgensen, S.E., Nielsen, S.N. & Mejer, H. 1995. Energy, environ, exergy and ecological modelling. *Ecol. Model.*, 77: 99-109.

Jørgensen, S.E. & Padisak, J. 1996. Does the intermediate disturbance hypothesis comply with thermodynamics? *Hydrobiologia*, 323: 9-21.

Jørgensen, S.E. & Bendoricchio, G. 2001. *Fundamentals of Ecological Modelling*, Elsevier, 530 p.

Jørgensen, S.E. & Marques, J.C. 2001. Thermodynamics and ecosystem theory. Case studies from Hydrobiology. *Hydrobiologia*, 445: 1-10.

Jørgensen, S.E., Marques, J.C. & Nielsen, S.N. 2002. Structural changes in an estuary, described by models and using Exergy as orientor. *Ecol. Model.*,158:233-240.

Jørgensen, S.E., Ladegaard, N., Debeljak, M. & Marques, J.C. 2005. Calculations of Exergy for organisms. *Ecol. Model.*, 185: 165-175.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. *Fisheries*, 6(6): 21-27.

Kimberling, D., Karr, J.R. & Fore, L.S. 2001. Measuring human disturbance using terrestrial in vertebrates in the shrub-steppe of eastern Washington (USA). *Ecol. Indic.*, 1: 63-81.

Koskimies, P. 1989. Birds as a tool in environmental monitoring. *Ann. Zool. Fenn.,* 6: 153-166.

Kryazheva, N.G., Chistyakova, E.K. & Zakharov, V.M. 1996. Analysis of development stability of *Betula pendula* under conditions of chemical pollution. *Russ. J. Ecol.*, 27: 422-424.

Kurelec, B., Kezic, N., Singh, H. & Zahn, R.K. 1984. Mixed-function oxidases in fish: Their role in adaptation to pollution. *Responses of Marine Organisms to Pollutants*, 14(1-4): 409-411.

Lafaurie, M., Mathieu, A., Salaun, J.P., Narbonne, J.F., Galgani, F., Romeo, M., Monod, J.L. & Garrigues, P.H. 1993. Biochemical markers in pollution assessment: Field studies along the North coast of the Mediterranean Sea. *Map. Tech. Rep. Ser.*, 69: 1-123.

Lambshead, P.J., Platt, H.M. & Shaw, K.M. 1983. The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *J. Nat. Hist.*, 17: 859-847.

Lambshead, P.J. & Platt, H.M. 1985. Structural patterns of marine benthic assemblages and their relationship with empirical statistical models. In: Gibbs, P.E. (Ed.), *Proceedings of the Nineteenth European Marine Biology Symposium*: Plymouth, 16-21.

Langston, W.J., Burt, G.R. & Zhou, M.J. 1987. Tin and organotin in water, sediments and benthic organisms of Poole Harbour. *Mar. Pollut. Bull.*, 18(12): 634-639.

Lauenstein, G., Robertson, A. & O'Connor, T. 1990. Comparison of trace metal data in mussels and oysters from a Mussel Watch programme of the 1970s with those from a 1980s programme. *Mar. Pollut. Bull.*, 21(9): 440-447.

Leary, R.F. & Allendorf, F.W. 1989. Fluctuating asymmetry as an indicator of stress in conservation biology. *Trends Ecol. Evol.*, 4: 214-217.

Leonard, D.R., Clarke, K.R., Somerfield, P R. & Warnick, R. M. (in press). The application of an indicator based on taxonomic distinctness for UK marine biodiversity assessment. *J. Environ. Manage.*

Leonzio, C., Bacci, E., Focardi, S. & Renzoni, A. 1981. Heavy metals in organisms from the northern Tyrhenian sea. *Sci. Total Environ.*, 20(2): 131-146.

Leppäkoski, E. 1975. Assessment of degree of pollution on the basis of macrozoobenthos in marine and brakish water environments. *Acta. Acad. Aba.,* 35: 1-98.

Levine, H.G. 1984.The use of seaweeds for monitoring coastal waters. In: Shubert, L.E. (Ed.), *Algae as Ecological Indicators*. Academic Press, London, 434 p.

Lewin, B. 1994. *Genes*. V. Oxford University Press, Oxford, 620 p.

Losovskaya, G.V. 1983. On significance of polychaetes as posible indicators of the Black Sea environment quality. *Ekologiya moray Publ.*, 12: 73-78.

Louette, M., Bijnens, L., Upoki Agenong'A, D. & Fotso, R.C. 1995. The utility of birds as bioindicators: case-studies in Equatorial Africa. *Belg. J. Zool.,* 125: 157-165.

Maurer, D., Leathem, W. & Menzie, C. 1981.The Impact of Drilling Fluid and Well Cuttings on Polychaete Feeding Guilds From the US Northeastern Continental Shelf. *Mar. Pollut. Bull.*, 12(10): 342-347.

McElroy, A.E. 1988. Trophic transfer of PAH and metabolites (fish, worm). *Responses of Marine Organisms to Pollutants*, 8(1-4): 265-269.

McGeoch, M.A. 1998. The selections, testing and application of terrestrial insects as bioindicators. *Biol. Rev.*, 73: 181-201.

McGinty, M. & Linder, C. 1997. An estuarine index of biotic integrity for Chesapeake Bay tidal fish community. In: Hartwell, S. (Ed.), *Biological Habitat Quality Indicators for Essential Fish Habitat.* NOAA Technical Memorandum NMFS-F/SPO-32, 124 p.

McIntyre, S., Lavorel, S. & Tremont, R.M. 1995. Plant life-history attributes: their relationship to disturbance in herbaceous vegetation. *J. Ecol.*, 83: 31-44.

McIver, J.D., Parsons, G.L. & Moldenke, A.R. 1992. Litter spider succession after clear-cutting in a western coniferous forest. *Can. J. Forestry Res.*, 22: 984-992.

McLachlan, S.M. & Bazely, D.R. 2001. Recovery patterns of understorey herbs and their use as indicators of deciduous forest regeneration. *Conserv. Biol.*, 15: 98-110.

McManus, J.W. & Pauly, D. 1990. Measuring ecological stress: Variations on a theme by R.M. Warwick. *Mar. Biol.*, 106 (2): 305-308.

Meire, P.M. & Dereu, J. 1990. Use of the abundance/biomass comparison method for detecting environmental stress: Some considerations based on intertidal macrozoobenthos and bird communities. *J. Appl. Ecol.*, 27(1): 210-223.

Mendez-Ubach, N. 1997. Polychaetes inhabiting soft bottoms subjected to organic enrichment in the Topolobampo lagoon complex, Sinaloa, México. *Océanides*, 12(2): 79-88.

Miller, B.S. 1986. Trace metals in the common mussel *Mytilus edulis* (L.) in the Clyde Estuary. *Proc. R. Soc. Edinb.*, 90: 379-391.

Milovidova, N.Y. & Alyomov, S.V. 1992. Physical/chemical characteristics of bottom sediments and soft bottom zoobenthos of Sevastopol region. In: Polykatpov, G. (Ed.), *Molysmology of the Black Sea*. Naukova Dumka, Kiev, 216-282 pp.

Mo, C. & Neilson, B. 1991. Variability in measurements of zinc in oysters, *C. virginica*. *Mar. Pollut. Bull.*, 22(10): 522-525.

Moffatt, S.F. & McLachlan, S.M. 2004. Understorey indicators of disturbance for riparian forests along an urban-rural gradient in Manitoba. *Ecol. Indic.*, 4: 1-16.

Mohlenberg, F. & Riisgard, H.U. 1988. Partioning of inorganic and organic mercury in cockles *Cardium edule* (L.) and *C.glaucum* (Bruguiere) from a chronically polluted area: influence of size and age. *Environ. Pollut.*, 55: 137-148.

Möller, A.P. 1998. Developmental instability of plants and radiation from Chernobyl. *Oikos,* 81: 444-448.

Möller, A.P. & Swaddle, J.P. 1997. Asymmetry, Developmental Stability, and Evolution. Oxford University Press, Oxford.

Molvær, J., Knutzen, J., Magnusson, J., Rygg, B., Skei, J & Sørensen, J. 1997. *Classification of environmental quality in fjords and coastal waters*. SFT guidelines. 97:03, 36 p.

Monaco, M.E. & Ulanowicz, R.E. 1997. Comparative ecosystem trophic structure of three U.S. mid-Atlantic estuaries. *Marine Ecology Progress Series*, 161: 239-254.

Moore J.C. & de Ruiter, P.C. 1991 Temporal and spatial heterogeneity of trophic interactions within belowground food webs. *Agriculture, Ecosystems and Environment*, 34, 371-397.

Moore J.C., de Ruiter, P.C. & Hunt, H.W. 1993. Influence of productivity on the stability of real and model ecosystems. *Science*, 261, 906-908.

Moore J.C. & de Ruiter, P.C.1997a. A food web approach to disturbance and ecosystem stability. In: *Ecological Principles for Risk Assessment of Contaminants in Soil* (eds. N.M. van Straalen and H. Løkke). Chapman & Hall.

Moore J.C. & de Ruiter, P.C. 1997 b. Resource compartmentation and the stability of real ecosystems. In: *Multitrophic interactions in terrestrial ecosystems* (eds. A.C. Gange and V. Brown) Blackwell Scientific Publications, Oxford.

Moreno, D., Aguilera, P.A. & Castro, H. 2001. Assessment of the conservation status of seagrass (*Posidonia oceanica*) meadows: implications for monitoring strategy and the decision-making process. *Biol. Cons.*, 102: 325-332.

Morrison, M.L. 1986. Bird populations as indicators of environmental changes. *Curr. Ornith.,* 3: 429–451.

Muxika, I., Borja, Á. & Franco, J. 2003. The use of a biotic index (AMBI) to identify spatial and temporal impact gradients on benthic communities in an estuarine area. *ICES CM 2003/ Session J-01*, Tallinn (Estonia), 24-28 September, 2003.

Muxika, I., Borja, Á. & Bald, J. (submitted) Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive.

Myers, M.S., Rhodes, L.D. & McCain, B.B. 1987. Pathologic anatomy and patterns of occurrence of hepatic neoplasm, putative preneoplastic lesions and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington, U.S.A. J. *Natl. Cancer Inst.*, 78(2): 333-363.

Nash, M.S., Whitford, W.G., Van Zee, J. & Havstad, K. 1998. Monitoring changes in stressed ecosystems using spatial patterns of ant communities. *Environ. Monit. Assess.*, 51: 201--210.

NEAGIG. 2004. Minutes of Northeast Atlantic Geographical Intercalibration Group Benthic Expert Meeting. Kristineberg Marine Station, Fiskebäckskil, Sweden, 22-24 September 2004.

Nelson, W.G. 1990. Prospects for development of an index of biotic integrity for evaluating habitat degradation in coastal systems. *Chem. Ecol.*, 4: 197-210.

Neumann, G. 1992. Responses of foraging ant population to high intensity wildfire, salvage logging, and natural regeneration processes in *Eucalyptus regnans* regrowth forest of the Victorian central highlands. *Austr. Forestry,* 55: 29-38.

Neumann, G., Notter, M & Dahlgaard, H. 1991. Bladder-wrack (*Fucus vesiculosus* L) as an indicator for Radionclides in the environment of Swedish nuclear power plants. *Swedish Environmental Protection Agency,* 3931: 1-35.

Nicholson, S. & Hui, Y.H. 2003. *Ecological monitoring for uncontaminated mud disposal investigation. First monitoring report, East of Ninepins*. Civil Engineering Department, Fill Management Division, The Government of Hong Kong Special Administrative Region, Mouchel Asia Environment, annexes. Unpublished Report, 34 p.

Niell, F.X. & Pazó, J.P. 1978. Incidencia de vertidos industriales en la estructura de poblaciones intermareales. II. Distribución de la biomasa y de la diversidad específica de comunidades de macrófitos de facies rocosa. *Inv. Pesq.,* 42(2): 231-239.

Nielsen, S.N. 1990 Application of exergy in structural-dynamical modelling. *Vehr. Int. Ver. Limnol.*, 24: 641-645.

Nielsen, S.N. 1992. Strategies for structural dynamics modelling. *Ecol. Model.*, 63: 91-101.

Nielsen, S.N. 1994. Modelling structural dynamic changes in a Danish Shallow Lake. *Ecol. Model.*, 73: 13-30.

Nielsen, S.N. 1995. Optimisation of exergy in a structural dynamic model. *Ecol. Model.*, 77:111-112.

Niemela, J., Langor, D. & Spence, J.R. 1993. Effects of clear-cut harvesting on boreal ground-bettle assemblages (Coleoptera : Carabidae) in western Canada. *Conser. Biol*, 7: 551-561.

Niencheski, L.F. 1982. Use of *Mytilus galloprovincialis* as pollution indicator in the French Mediterranean coast. Chlorinated organic compounds and heavy metals. *Atlantica*, 5 (2): 85-86.

Niquil, N., Arias-González, J.E., Delesalle, B. & Ulanowicz, R.E. 1999. Characterization of the planktonic food web of Takapoto Atoll lagoon, using network analysis. *Oecologia*, 118: 232-241.

O'Connor, J.S. & Dewling, R.T. 1986. Indices of marine degradation: their utility. *Environ. Manage.*, 10: 335-343.

Ohlson, M., Söderström, L., Hörnberg, G., Zackrisson, O. & Hermansson, J. 1997. Habitat qualities versus long-term continuity as determinants of biodiversity in Boreal old-growth swamp forests. *Biol. Cons.*, 81: 221–231.

Orfanidis, S., Panayotidis, P. & Stamatis, N. 2001. Ecological evaluation of transitional and coastal waters: a marine benthic macrophytes-based model. *Mediterranean Mar. Sci.*, 2: 45-65.

Orfanidis, S., Panayotidis, P. & Stamatis, N. 2003. An insight to the ecological evaluation index (EEI). *Ecol. Indic.*, 3: 27-33.

OSPAR/MON. 1998. *Report of ad hoc working group on monitoring* (MON), Copenhagen, 23-27 February 1998.

Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. In: Markow, T.A. (Ed.), *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer Academic Publishers, Netherlands, 335–364 pp.

Palmer, A.R. & Strobeck, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annu. Rev. Ecol. Syst.*, 17: 391–421.

Pardal, M.A., Marques, J.C., Metelo, I., Lillebø, A.I. & Flindt, M.R. 2000. Impact of eutrophication on the life cycle, population dynamics and production of *Ampithoe valida* (Amphipoda) along an estuarine spatial gradient (Mondego estuary, Portugal). *Mar. Ecol. Prog. Ser.*, 196: 207-219.

Pardal, M.A., Cardoso, P.G., Sousa, J.P., Marques, J.C. & Raffaelli, D. 2004. Assessing environmental quality: a novel approach. *Mar. Ecol. Prog. Ser.*, 267: 1-8.

Patrício, J., Ulanowicz, R.E., Pardal, M.A. & Marques, J.C. 2004. Ascendency as ecological indicator: A case study on estuarine pulse eutrophication. *Estuar. Coast. Shelf Sci.*, 60(1): 23-35.

Patrício, J., Salas, F., Pardal, M.A., Marques, J.C. & Jørgensen, S.E. 2006. Ecological indicators in a re-colonisation field experiment. Contribution to theoretical ecology. *Ecological Indicators*, 6: 43-57.

Pearson, T.H. & Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev.*, 16: 229-331.

Pérez-Ruzafa, A., Ros, J.D., Marcos, C., Ballester, R. & Pérez-Ruzafa, I.M. 1989. Distribution and biomass of the macrophyte beds in a hypersaline coastal lagoon (the Mar Menor, SE Spain), and its recent evolution following major environmental changes. In: Boudoresque, C.F., Meinenesz, A., Fresi, E. & Gravez, V. (Eds.), *International Workshops on Posidonia Beds*. GIS Posidonie Publications, France, 2:49-62.

Pérez-Ruzafa, A. & Marcos, C. 1992. Colonization rates and dispersal as essential parameters in the confinement theory to explain the structure and horizontal zonation of lagoon benthic assemblages. *Rapp. Comm. int. Mer Medit.,* 33: 100

Pérez-Ruzafa, A., Aliaga, V., Barcala, E., García-Charton, J.A., González-Wangúemert, M., Gutiérrez-Ortega, J.M., Marcos C. & Salas, F. 1994. Caracterización y valoración del medio marino que constituye el entorno físico y biótico de la central térmica de Escombreras. Documento científico restringido. Universidad de Murcia.

Pérez-Ruzafa, A., Aliaga, V., Marcos, C. & Salas, F. 1997. Seguimiento del impacto producido por las jaulas suspendidas para engorde de atunes sobre las comunidades bentónicas del área de Cabo Tiñoso. Documento científico restringido. Universidad de Múrcia.

Pérez-Ruzafa, A., Marcos, C. & Gilbert, J. 2005. The ecology of the Mar Menor coastal lagoon: a fast changing ecosystem under human pressure. In: Gönenc, J.E., & Wolflin, J. (Eds.), *Coastal lagoons: ecosystem processes and modelling for sustainable use and development*. CRC Press, Boca Raton, 392-422 pp.

Pérez-Ruzafa, I. 2003. Efecto de la contaminaciónsobre la vegetación submarina y su valor indicador. In: Pérez-Ruzafa, A., Marcos, C., Salas, F & Zamora, S (eds). *Perspectivas y herramientas en el estudio de la contaminación marina*. Servicio de Publicaciones, Universidad de Murcia. Murcia, 133-147 pp.

Pergent-Martini, C. 1998. *Posidonia oceanica*: a biological indicator of past and present mercury contamination in the Mediterranean sea. *Mar Environ Res* 45: 101-111.

Pergent-Martini, C., Leoni, V., Pasqualini V., Ardizzone , G.D., Balestri ,E., Bedini, R., Belluscio, A., Belsher, T., Borg, J., Boudouresque, C.F., Boumaza, S., Bouquegneau, J.M., Buia, M.C., Calvo, S. *et al*. 2005. Descriptors of *Posidonia oceanica* meadows: Use and Application. *Ecol. Indic*., 5: 213-230.

Petrov, A.N. 1990. Study on ecology of molluscs (the Black Sea Bivalves) employing some relevant indices. PhD Thesis, Sevastopol, 286 p.

Petrov, A.N. & Shadrina, L.A. 1996. The assessment of impact of the Tchernaya river estuary and municipal sewages on state of marine communities in Sevastopol bay (the Black Sea). In: *Estuarine Environments and Biology of estuarine species*. Crangon publ., Gdansk, 161--169 pp.

Philips, D.H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - A review. *Environ. Pollut*., 13: 281-317.

Picard-Berube, M. & Cossa, D. 1983. Teneurs en benzo 3,4 pyréne chz *Mytilus edulis*. L. de léstuarie et du Golfe du Saint-Laurent. *Mar. Environ. Res*., 10: 63-71.

Pielou, E.C. 1969. *An Introduction to Mathematical Ecology*. Wiley Interscience, New York, 286 p.

Pires, S. & Muniz, P. 1999. Trophic structure of polychaetes in the Sao Sebastiao Channel (southeastern Brazil). *Mar. Biol*., 5: 517-528.

Plafkin, J.L., Barbour, M.T., Porte, K.D., Gross, S.K. & Hughes, R.M. 1989. Rapid bioassessment protocols for use in streams and rivers. Benthic macroinvertebrates and Fish. EPA/444/4-89/001. Office and Water Regulations and Standards, US. Environmental protection Agency, Washington, DC.

Planas, M. & Mora, J. 1987. Estado de conocimiento actual del bentos en zonas organicamente enriquecidas. *Thalassas*, 5(1):125-134.

Pocklington, P., Scott, D.B. & Schafer, C.T. 1994. Polychaete response to different aquaculture activities. *Proceedings of the 4th International Polychaete Conference*, 162: 511-520.

Price, P.W. 1988. An overview of organismal interactions in ecosystems in evolutionary and ecological time. *Agric. Ecosyst.Environ.,* 24: 369–377.

Prior A., Miles, A.C., Sparrow, A.J. & Price, N. 2004. Development of a classification scheme for the marine benthic invertebrate component, Water Framework Directive. Phase I & II - Transitional and coastal waters. R & D Interim Technical Report E1-116/E1-132, 103 p.

Purtauf, T., Dauber, J. & Wolters, V. 2005. The response of carabids to landscape simplification differs between trophic groups. *Oecologia*, 142: 458-464.

Raffaelli, D.G. & Mason, C.F. 1981. Pollution monitoring with meiofauna using the ratio of nematodes to copepods. *Mar. Poll. Bull.,* 12: 158-163.

Ramm, A. E. 1988. The Community Degradation Index: A new method for assessing the deterioration of aquatic habitats. *Water Res.*, 22: 293-301.

Ramm, A. E. 1990. Application of the community degradation index to South African estuaries. *Water Res.*, 24: 383-389.

Raposo, P. 1996. Biología e Ecologia de *Liza ramada* (Risso, 1826) e *Chelon labrosus* (Risso, 1826) (Pises, Mugilidae). Inter-relações com o ecosistema estuarino. PhD Thesis, University of Lisbon.

Refseth, D. 1980. Ecological analyses of carabid communities-potential use in biological classification for nature conservation. *Biol. Cons.*, 17: 131-141.

Regoli, F. & Orlando, E. 1993. *Mytilus galloprovincialis* as a bioindicator of lead pollution: Biological variables and cellular responses. In: Sloof, W. & De Kruijf, H. (Eds.), *Proceedings of the Second European Conference on Ecotoxicology*: 1-2 .

Reish, D.J. 1993. Effects of metals and organic compounds on survival and bioaccumulation in two species of marine gammaridean amphipod, together with a summary of toxicological research on this group. *J. Nat. Hist.*, 27: 781-794.

Reish, D.J. & Gerlinger, T.V. 1984. The effects of cadmium, lead, and zinc on survival and reproduction in the polychaetous annelid *Neanthes arenaceodentata* (F. Nereididae). *Proceedings of the First International Polychaete Conference:* 383-389.

Reizopoulou, S. Thessalou-Legaki, M. & Nicolaidou, A. 1996. Assesment of disturbance in Mediterranean lagoons: an evaluation of methods. *Mar. Biol.*, 125: 189-197.

Renberg, L., Tarkpea, M. & Sundstroem, G. 1986. The use of the bivalve *Mytilus edulis* as a test organism for bioconcentration studies: II. The bioconcentration of two super(14)C-labelled chlorinated paraffins. *Ecotoxicol. Environ. Saf.*, 11(3): 361-372.

Resh, V.H & Jackson, J.K. 1993. Rapid assessment approaches to biomonitoring using benthic invertebrates, In: Rosenberg, D.M. & Resh, V.H. (Eds.), *Freswater biomonitoring and benthic invertebrates*. Chapman & Hall. NY., 194-219 pp.

Reynaud, P.A. & Thiolouse, J. 2000. Identification of birds as biological markers along a neotropical urban-rural gradient (Cayenne, French Guiana) using co-inertia analysis. *J. Environ. Manage.*, 59: 121-140.

Richardson, B.J. & Waid, J.S. 1983. Polychlorinated biphenyls (PCBs) in Shellfish from Australian coastal waters. *Ecol. Bull. (Stockholm),* 35: 511-517.

Riisgard, H.U., Kierboe, T., Molenberg, F., Drabaek, I. & Madsen, P. 1985. Accumulation, elimination and chemical speciation of mercury in the bivalves *Mytilus edulis* and *Macoma balthica. Mar. Biol.,* 86: 55-62.

Ringwood, A.H., Brouwer, M., Forlin, L. & Andersson, T. 1995. Patterns of metallothionein expression in oyster embryos. *Mar. Environ. Res.*, 39(1/4): 101-105.

Ritz, D.A., Swain, R. & Elliot, N.G. 1982. Use of the mussel *Mytilus edulis planulatus* (Lamarck) in monitoring heavy metal levels in seawater. *Aust. J. Mar. Freshwater Res.*, 33(3): 491-506.

Ritz, D.A., Lewis, M.E. & Shen, M. 1989. Response to organic enrichment of infaunal macrobenthic communities under salmonid seacages. *Mar. Biol.*, 103: 211-214.

Roberts, R.D., Gregory, M.G. & Foster, B.A. 1998. Developing an efficient macrofauna monitoring index from an impact study-a dredge spoil example. *Mar. Pollut. Bul.*, 36(3):231-235.

Robinson, G.R., Yurlina, M.E. & Handel, S.N. 1994. A century of change in the Staten Island flora: ecological correlates of species losses and invasions. *Bull. Torrey Bot. Club.*, 121: 119-129.

Roesijadi, G. 1994. Metallothionein induction as a measure of response to metal exposure in aquatic animals. *Genetic and Molecular Ecotoxicology* , 102(12): 91-96.

Romeo, M. & Gnassia-Barelli, M. 1988. *Donax trunculus* and *Venus verrucosa* as bioindicators of trace metal concentrations in Mauritanian coastal waters. *Mar. Biol.*, 99(2): 223-227.

Romero, J., Alcoverro, T., Martínez-Crego, B. & Pérez, M. 2005. The seagrass *Posidonia oceanica* as a quality element under the Water Framework Directive: POMI, a multivariate method to assess ecological status of Catalan coastal waters. Working document of the POMI group, Universitty of Barcelona and Centre d'Estudis Avançats de Blanes-CSIC.

Ros, J.D., Cardell, M.J., Alva, V., Palacin, C & Llobet, I. 1990. Comunidades sobre fondos blandos afectados por un aporte masivo de lodos y aguas residuales (litoral frente a Barcelona, Mediterráneo occidental): resultados preliminares. *Bentos*, 6: 407-423.

Ros, J.D. & Cardell, M.J. 1991. La diversidad específica y otros descriptores de contaminación orgánica en comunidades bentónicas marinas. *Actas del Symposium sobre Diversidad Biológica.* Centro de Estudios Ramón Areces, Madrid, 219-223 pp.

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

Ross, L.T. & Jones, D.A. 1979. Biological aspects of water quality in Florida. Technical series, vol 4 (3). Department of Envronmental regulation, State of Florida, Tallahese, FL.

Ruiz, J.L., Perez, M. R Romero, J. 2001. Effects of fish farm loadings on seagrass (*Posidonia oceanica*): distribution, growth and photosynthesis. *Mar. Pollut. Bull.*, 42(9): 749-760

Rygg, B. 1985. Distribution of species along a pollution gradient induced diversity gradients in benthic communities in Norwegian Fjords. *Mar. Pollut. Bull.,* 16(12): 469-473.

Rygg, B. 2002. Indicator species index for assessing benthic ecological quality in marine waters of Norway. *Norwegian Institute for Water Research,* Report Nº 40114, 1-32 pp.

Salas, F. 2002. *Valoración y aplicabilidad de los índices y bioindicadores de contaminación orgánica en la gestión del medio marino*. PhD Thesis, University of Murcia, Spain.

Salas, F., Neto, J.M., Borja, Á. & Marques, J.C. 2004. Evaluation of the applicability of a Marine Biotic Index to characterise the status of estuarine ecosystems: The case of Mondego estuary (Portugal). *Ecol. Indic.*, 4: 215-225.

Salas, F., Marcos, C., Pérez-Ruzafa, A. & Marques, J.C. 2005. Application of the Exergy Index as ecological indicator along an organic enrichment gradient in the Mar Menor Lagoon (South-Eastern Spain). *Energy*, 30: 2505-2522.

Sanders, H.L. 1968. Marine benthic diversity: a comparative study. *Am. Nat.*, 102: 243-282.

Satsmadjis, J. 1982. Analysis of benthic fauna and measurement of pollution. *Rev. Int. Oceanogr. Med.*, 66-67:103-107.

Satsmadjis, J. 1985. Comparison of indicators of pollution in the Mediterranean. *Mar. Pollut. Bull.*, 16: 395-400.

Shackleford, B. 1988. *Rapid bioassiessments of lotic macroinvertebrate communities: biocriteria development*. Arkansas Dept. of Pollution Control and Ecology, Little Rock, Arkansas.

Shannon, C.E. & Weaver, W. 1963. *The Mathematical theory of communication*. University of Illinois Press.

Shaw, K.M., Lambshead, P.J.D. & Platt, H.M. 1983. Detection of pollution-induced disturbance in marine benthic assemblages with special reference to nematodes. *Mar. Ecol. Prog. Ser.*, 11: 195-202.

Simboura, N. & Zenetos, A. 2002. Benthic indicators to use ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index. *Mediterranean Mar. Sci.*, 3/2: 77-111.

Simpson, E.H. 1949. Measurement of diversity. *Nature*, 163: 688.

Smith, R.W., Bergen, M., Weisberg, S.B., Cadien, D., Dalkey, A., Montagne, D., Stull., J.K & Velarde, R.G. 2001. Benthic response index for assessing infaunal communities on the Mainland shelf of southern California. *Ecol. Appl.*,11: 1073-1087.

Somerfield, P.J., Olsgard, F. & Carr, M.R. 1997. A further examination of two new taxonomic distinctness measures. *Mar. Ecol. Prog. Ser.*, 154: 303-306.

Somerfield, P.J & Clarke, K.R. & Warwick, R.M. 2003. Taxonomic distinctness as an indicator of biodiversity and environmental health: analysis of data and draft recommendations. Plymouth Marine Laboratory, Plymouth.

Spies, R.B., Felton, J.S. & Dillard, L. 1984. Hepatic mixed-function oxidases in California Flatfishes are increased in contaminated environments and by oil and PCB ingestion. *Response of marine organisms to pollutants*, 14(1-4): 412-413.

Spitzer, K., Jaros, J., Havelka, J. & Leps, J. 1997. Effects of small-scale disturbance on butterfly communities of an Indochinese montane rainforest. *Biol. Conserv.*, 80: 9-15.

Stewart, P.L. 1994. Environmental requirements of blue mussel (Mytilus edulis) in eastern Canada and its response to human impacts. *Can. Tech. Rep. Fish. Aquat. Sci.*, 3: 1-54.

Stirn, J., Avcin, A.J., Kerzan, I., Marcotte, B.M., Meith-Avcin, N., Vriser, B. & Vukovic, S. 1971. Selected biological methods for assessment of pollution. In: Pearson, E.A. & Defraja, E. (Eds.), *Marine pollution and waste disposal*. Pergamon Press, Oxford, 307-328 pp.

Storelli, M.M & Marcotrigiano, G.O. 2001. Persistent organochlorine residues and toxic evaluation of polychlorinated biphenyls in sharks from the Mediterranean Sea (Italy). *Mar. Pollut. Bull.,* 42(12): 1323-1329.

Straškraba, M. 1983. Cybernetic formulation of control in ecosystems. *Ecol. Model.*, 18: 85-98.

Szaro, R.C. 1986. Guild management: an evaluation of avian guilds as a predictive tool. *Environ. Manage.*, 10: 681-688.

Taghon, G.l., Nowell, A.R.. & Jumars, P. 1980. Induction of suspension feeding in Spionid Polychaetes by high particulate fluxes. *Science*, 210: 562-564.

Uetz, G.W. 1976. Gradient analysis of spider communities in a streamside forest. *Oecologica*, 22: 373-385.

Ulanowicz, R.E. 1980. An hypothesis on the development of natural communities. *J. Theor. Biol.*, 85: 23-245.

Ulanowicz, R.E. 1986. *Growth and development ecosystems phenomenology*. Springer-Verlag, New York.

Ulanowicz, R.E. 1997. Ecology, the Ascendent Perspective. Columbia University Press, New York, 201 pp.

Ulanowicz, R.E. 2000. Ascendency: a measure of ecosystem performance. In: S.E Jørgensen & F. Müller (eds), Handbook of Ecosystem Theories and Management. Lewis Publishers, Boca Raton, 303-315 p.

Ulanowicz, R.E. & Norden, J.S. 1990. Symmetrical overhead in flow and networks. *Int. J. Syst. Sci.*, 21(2): 429-437.

Ulanowicz, R.E. & Wulff, F. 1991. Comparing ecosystem structures: the Chesapeake Bay and the Baltic Sea. In: J. Cole, G. Lovett & S. Findlay (eds), Comparative Analyses of Ecosystems: Patterns, Mechanisms and Theories. Spring Verlag, New York, 140-166 p.

UNESCO. 2003. A reference guide on the use of indicators for integrated coastal management. ICAM Dossier 1, IOC Manuals and Guides, 45 p.

Vaas, P.A. & Jordan, S.J. 1990. Long term trends in abundance indices for 19 species of Chesapeake Bay fishes: reflection of trends in the Bay ecosystem. In: *New perspectives in the Chesapeake System: a Research and Management Partnership*. Proceedings of a Conference.Chesapeake Research Consortium Publ. 137.

Van der Pijl, L. 1972. *Principles of Dispersal in Higher Plants*. Springer-Verlag, Berlin.

Van Dolah, R.F., Hyland, J.L., Holland, A.F., Rosen, J.S. & Snoots, T.R. 1999. A benthic index of biological integrity for assessing habitat quality in estuaries of the southeastern USA. *Mar. Environ. Res.*, 48: 269-283.

Varanasi, U., Chan, S. & Clark, R. 1989. *National Benthic Surveillance Project: Pacific coast.* National Ocean Service. Washington.

Verlaque, M. 1977. Impact du rejet thermique de Martigues-Ponteau sur le macrophytobenthos. *Tethys,* 8(1): 19-46.

Viarengo, A. & Canesi, L. 1991. Mussels as biological indicators of pollution. *Aquaculture*, 94: 225-243.

Villalba, A. & Vieitez, J.M. 1985. Estudio de la fauna de anélidos poliquetos del sustrato rocoso intermareal de una zona contaminada de la Ría de Pontevedra (Galicia). *Cah. Biol. Mar.*, 26: 359-377.

Warwick, R.M. 1986. A new method for detecting pollution effects on marine macrobenthic communities. *Mar. Biol.*, 92: 557-562.

Warwick, R.M. 1993. Environmental impact studies on marine communities: Pragmatical considerations. *Aust. J. Ecol.*, 18: 63-80.

Warwick, R.M. & Clarke, K.R. 1994. Relearning the ABC: taxonomic changes and abundance/biomass relationships in disturbed benthic communities. *Mar. Biol.*, 118: 739-744.

Warwick, R.M. & Clarke K.R. 1995. New ‘biodiversity’ measures reveal a decrease in taxonomic distinctness with increasing stress. *Mar. Ecol. Prog. Ser.*, 129:, 301-305.

Warwick, R.M. & Clarke, K.R. 1998. Taxonomic distinctness and environmental assessment. *J. Appl. Ecol.*, 35: 532-543.

Warwick, R.M. & Clarke, K.R. 2001. Practical measures of marine biodiversity based on relatedness of species. *Oceanogr. Mari. Biol. Ann. Rev.*, 39: 207-231.

Weisberg, S.B., Ranasinghe, J.A., Dauer, D.M., Schaffner, L.C., Díaz, R.J & Frithsen, J.B. 1997. An estuarine benthic index of biotic integrity (B-IBI) for the Chesapeake Bay. *Estuaries*, 20: 149-158.

Weston, D.P. 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Mar. Ecol. Prog. Ser.*, 61(3): 233-244.

WGBEC. 2002. *Report of the Working Group on Biological effects of contaminants*. Murcia, 11-15 March 2002. ICES CM 2002/E: 02 Ref. ACME.

Wilsey, B.J., Haukioja, E., Koricheva, J. & Sulkinoja, M. 1998. Leaf fluctuating asymmetry increases with hybridization and elevation in tree-line birches. *Ecology,* 79: 2092–2099.

Wollenweider, R.A., Giovanardi, F., Montanari, G. & Rinaldi, A. 1998. Characterisation of the trophic conditions of marine coastal waters with special reference to the NW Adriatic Sea: Proposal for a trophic scale, turbidity and generalised water quality index. *Environmetrics*, 9: 329-357.

Wooton, M. & Lye, A.K. 1982. Metal levels in the mussel *Mytilus edulis* collected from estuaries in south eastern Australia. *Aust. J. Mar. Freshwater Res.*, 32(2): 363-367.

Word, J.Q. 1979. The infaunal trophic index. *Calif. Coast. Wat. Res. Proj. Annu. Rep.*, 19-39 pp.

Word, J.Q. 1980. The infaunal trophic index. The 1980 Annual Report, Southern California Coastal Research Project. Long Beach, CA, 19-39 pp.

Word, J.Q. 1990. The infaunal trophic index a functional approach to benthic community analyses. PhD Thesis. University of Washington, 297 p.

Yokoyama, H. 1997. Effects of fish farming on macroinvertebrates. Comparison of three localities suffering from hypoxia. *UJNR Technical Report.*, 24: 17-24.

Zabala, K., Romero, A. & Ibáñez, M. 1983. La contaminación marina en Guipuzcoa: I. Estudio de los indicadores biológicos de contaminación en los sedimentos de la Ría de Pasajes. *Lurralde*, 3: 177-189.

Zvereva, E.L., Kozlov, M.V., Niemelä, P. & Haukioja, E. 1997. Delayed induced resistance and increase in leaf fluctuating asymmetry as responses of *Salix borealis* to insect herbivory. *Oecologia,* 109: 368–373.

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