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Biotic indices for assessing the status of coastal waters: a review of strengths and weaknesses

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Biotic indices have become key assessment tools in most recent national and trans-national policies aimed at improving the quality of coastal waters and the integrity of their associated ecosystems. In this study we analyzed 90 published biotic indices, classified them into four types, and analyzed the strengths and weaknesses of each type in relation to the requirements of these policies. We identified three main type-specific weaknesses. First, the problems of applicability, due to practical and conceptual difficulties, which affect most indices related to ecosystem function. Second, the failure of many indices based on structural attributes of the community (*e.g.* taxonomic composition) to link deterioration with causative stressors, or to provide an early-detection capacity. Third, the poor relevance to the ecological integrity of indices based on attributes at the sub-individual level (*e.g.* multi-biomarkers). Additionally, most indices still fail on two further aspects: the broad-scale applicability and the definition of reference conditions. Nowadays, the most promising approach seems to be the aggregation of indices with complementary strengths, and obtained from different biological communities.

Environmental impact

Assessing the quality of coastal waters is a crucial issue for society. Performing this assessment using metric, comparable and transparent scales, internationally accepted and scientifically sound, is a major challenge for scientists and managers. The concept of water quality (and, hence, water management) has evolved into a much more holistic view for incorporating not only physico-chemical but also biological and ecological notions. Consequently, the design and implementation of bioindicators has become a major field in applied ecology, resulting in an exacerbated market of biotic indices. We revise here the offer of this market, and evaluate the strengths and weaknesses of the different possibilities from the point of view of the users' needs. In this way, we try to bring some light at the interface between science and society, from the point of view of the environment.

Perspectives in the current use of biotic indices in coastal waters

The coastal zone has historically played a crucial role in human life. A large proportion of the human population inhabits coastal areas,[1] and human density there is expected to increase in the coming years. Consequently, coastal ecosystems are particularly exposed to human pressures, and some of them are among the most disturbed parts of the biosphere.[2,3] Society and managers require tools based on sound scientific knowledge to properly monitor, manage and protect such sensitive areas.

The earliest studies of water quality assessment focused mainly on the water itself, and its quality was most often expressed in terms of physical and chemical parameters. This view is conceptually linked to point sources of pollution; however, non-point sources of pollution have been increasingly recognized as being responsible for many water quality problems.[4] Due to this new perception, together with a better understanding of the interconnection between ecosystem services and human welfare,[5] the concept of water quality (and, hence, water management) has evolved into a broader, more holistic approach, which incorporates biological and ecological criteria. Within this context, the ecological integrity of water bodies under human pressure has been defined as the ability of the aquatic ecosystem to support and maintain key ecological processes and a community of organisms with a species composition, diversity and functional organization similar to that of undisturbed habitats within the region.[4] Finding the causes of reduced aquatic system integrity, and developing and implementing adequate remedial actions are now key components of water quality management.

To a great extent, this approach is reflected in the large-scale (national and trans-national) strategies currently in force, such as the EU Water Framework Directive (WFD 2000/60/EC), the Environmental Monitoring and Assessment Program (EMAP 2002) derived from the US Clean Water Act (CWA), and the Australian and New Zealand Water Quality Management Strategy (WQMS 1992). All of the above are aimed at maintaining and improving the status of the Nation's or Member State's waters. To do that, they establish that the implementation of an effective and coherent water policy must address, as a key component of water quality, the integrity of the aquatic ecosystems. Consequently, the strategic importance of reliable, quantitative, and directly comparable methods for assessing the integrity of coastal aquatic ecosystems on a large scale has promoted an expanding body of research focused on the field of bioindicators and biotic indices (Fig. 1).

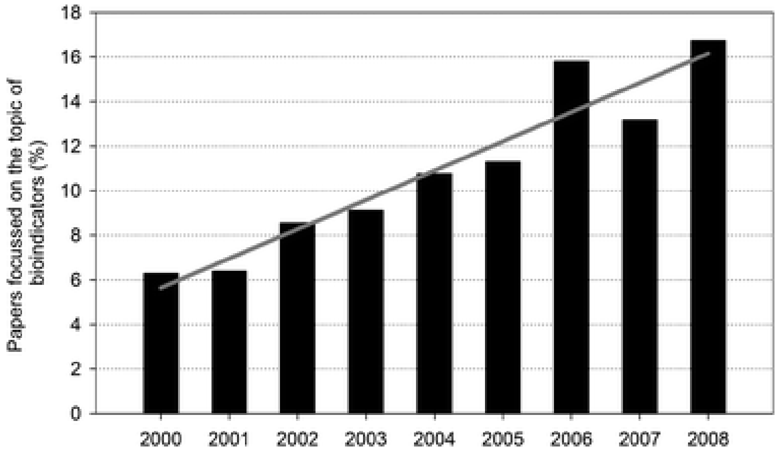


Fig. 1 Percentage of papers published in 96 journals from the subject areas of ecological, toxicological and environmental sciences between 2000 and 2008, searched by topic (biomarker, bioindicator and biotic index) and including terrestrial, freshwater and marine and estuarine systems. Search performed on the ISI Web of Knowledge database. The regression line shows the increasing trend.

A bioindicator is an organism, a part of an organism, or a set of organisms that contains information on the quality of the environment.[6] Bioindicators can be obtained from any level of the biological organization, ranging from the biochemistry or metabolism of a single organism to emergent properties of complex communities (Fig. 2). Biotic indices go one step further and attempt to summarize features of different elements of the ecosystem (several bioindicators, community level information) into a single value,[4] integrating relevant ecological information into an overall expression of biotic integrity.

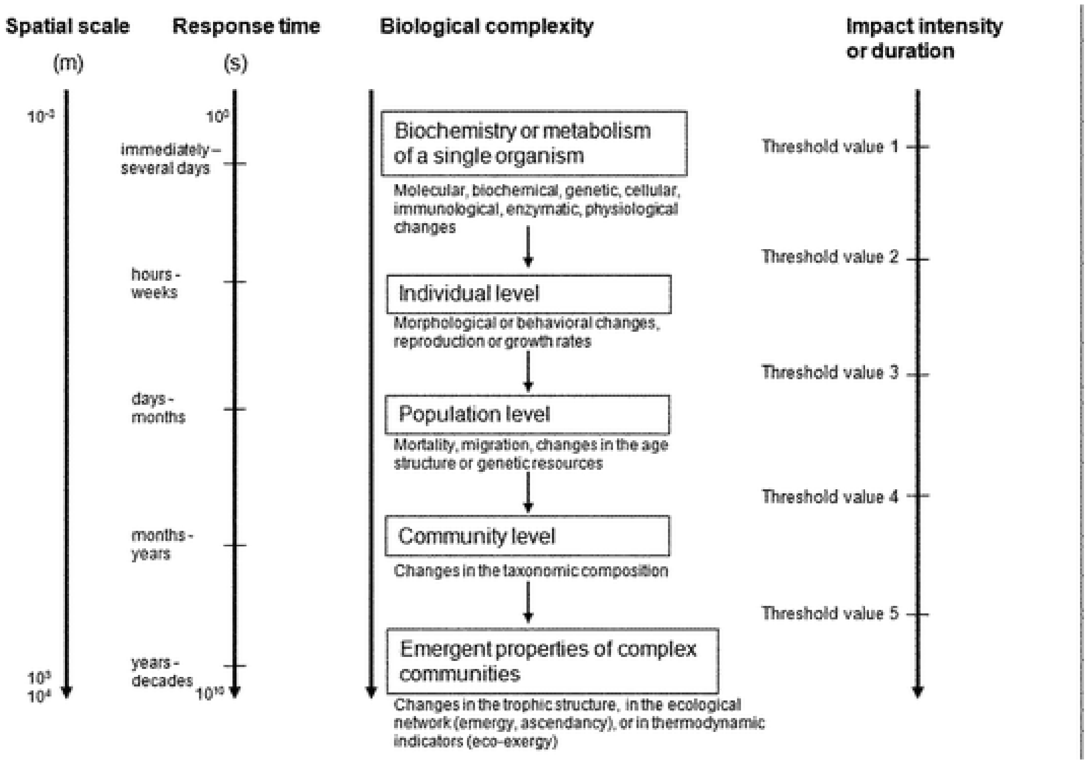


Fig. 2 Average stress response times of biotic systems as related to the biological system size and structural complexity, and to the impact intensity or duration (modified from Fränzle, 2006).[7]

Here, we review the scientific literature in order to establish the current state and future perspectives of research in the field of bioindicators and biotic indices, and in the context of large-scale assessments of coastal waters quality. In the first part of the paper, we analyze the features and properties necessary to fulfil the requirements derived from the above cited national and trans-national strategies for water quality management. Secondly, we present a compilation of 90 biotic indices proposed to date for marine and estuarine waters, classify them into four types, and analyze the type-specific strengths and weaknesses in relation to these requirements.

Requirements for biotic indices in large-scale water quality management strategies

The most recent large-scale strategies for preserving water quality have identified common bioindicator requirements, which can be drawn from the guidelines developed to put in force the US CWA,[8] the Australian WQMS,[9] and the European WFD.[10–12] These requirements can be summarized as: relevance to ecological integrity, broad-scale applicability, early-detection capacity, feasibility of implementation, interpretability against reference conditions, and capacity to link ecosystem degradation with its causative stressors.

Relevance to ecological integrity

Biological measures should be capable of reflecting the integrity of the entire ecosystem. Ideally, disturbance effects on the complete assemblage of organisms should be studied; however, a particular assemblage or a key component is often measured as being representative of the entire community. Phytoplankton, aquatic flora, benthic invertebrate fauna, and fish fauna are the most commonly proposed organisms for quality bioassessment programs of coastal and estuarine waters. The biological measures obtained from those organisms which most closely reflect the status and trends of the ecosystem concerned should be selected.

Broad-scale applicability

A key feature of the different strategies for water management is their large spatial scale applicability, usually in the order of thousands of kilometres. Many theoretical and practical difficulties arise when developing bioassessment tools for such broad-scale applicability. These are due to the high natural variability of biological systems (and consequently of bioindicators), together with the interactive effect of multiple human stressors potentially affecting them in a punctual or diffuse manner. With this in mind, the definition of bioindicator reference conditions for more or less homogeneous geographic areas (eco-regions) has been proposed for reducing the confounding effects of variability other than those caused by human pressure (*e.g.* geomorphology, climatic, *etc.*). This may contribute towards the development of a large-scale based definition of ecosystem integrity.

Early-detection capacity

The early detection of environmental deterioration is necessary for several reasons, whether economic, practical, ethical or strategic. When required, management actions should be implemented in time to prevent serious ecosystem damage, avoiding prolonged (and sometimes uncertain) recovery and/or costly remedial actions. Therefore, bioindicators should help to anticipate environmental problems before they become acute.

Feasibility of implementation

The bioassessment tools should be based on relatively widely distributed organisms, and should use standard protocols which do not present significant technical difficulties, as far as possible. To a certain extent, however, feasibility is contingent on many factors, as often it depends on a certain trade-off between the bioindicator requirements (robustness, specificity, spatial and temporal resolution), and the available resources (knowledge, staff, equipment, financial support).

Interpretability against reference conditions

The definition of reference conditions against which to compare the current ecosystem status has become common practice, helping to harmonize results. This definition depends on an unambiguous and non-arbitrary determination of the system structure and function. “Minimally or least disturbed condition”, “historical condition”, and “best attainable condition” obtained by extrapolation of empirical models can be used as standards or benchmarks against which to compare the current condition. The defined reference conditions allow the development of numerical methods that evaluate the ecosystem condition within a simple and broadly understandable range. The evaluation of adequate statistical confidence and precision in the assigned ecosystem condition, and of the probability of assigning a wrong class due to errors is far less common, although it would be a great help.

Linking ecosystem degradation to its causative stressors

Biological measures should be both sensitive to multiple stressors and, to a certain extent, specific enough to provide some clues about the possible causes of deterioration. Certainly, biotic indices alone will hardly identify unequivocally the agents responsible for an observed quality loss in coastal waters. However, managers not only need to be warned about status deterioration, but also would need insights to guide effective remedial actions to restore water quality. For this, some kind of diagnostic on causal factors will be of great help.

Biotic indices for coastal waters

Review methodology

We search by topic (biomarker, bioindicator and biotic index) on the ISI Web of Knowledge database for papers published in 96 journals from the subject areas of ecological, toxicological and environmental sciences between 2000 and 2008. Then, we select those indices that have been successfully applied in the context of the large-scale strategies, until we obtain a reasonably complete list of biotic indices covering the whole existent spectra of approaches. Finally, the search was enlarged to previous years in order to include the references that first cited and successfully applied them. We summarized 90 biotic indices designed for use in marine and/or estuarine habitats (Table 1), and critically evaluated their strengths and weaknesses in the light of the requirements previously identified (see above). Our review is not meant to be exhaustive, and we limit our discussion to the indices published only in peer-reviewed journals, and having been successfully applied within the context of these large-scale strategies. In doing so, we omit information about the original authors of some “classic” indices that were not validated within this context. Moreover, we recognize that some specific indices published in peer-reviewed journals or some methods published in the so-called “grey literature” may have been overlooked. However, we cover the whole existent spectra by analyzing sufficient biotic indices based on different biological systems, whose formulations encompass the most relevant approaches identified.

Some previous reviews have provided a more or less complete overview of the properties of diverse index typologies from a conceptual point of view.[13] Others have provided a description of diverse indices, although limited to or biased towards certain communities (*e.g.* fishes,[14] benthic macroinvertebrates[15–17]), and/or to certain types of indices (*e.g.* biotic indices based on non-taxonomy assessments of the community structure,[18] or indicators based on emergent properties[19]). These reviews mainly concluded that there are a large number of potential ecosystem status indicators, and that the challenge is to select the combined suite of indicators that will best serve user needs. Here, we contribute to this challenge by providing arguments for such a selection.

To facilitate the discussion of their respective strengths and weaknesses, we propose the following four types of biotic indices, which are based on the main approaches identified in the formulation of the 90 indices reviewed (see Fig. 3):

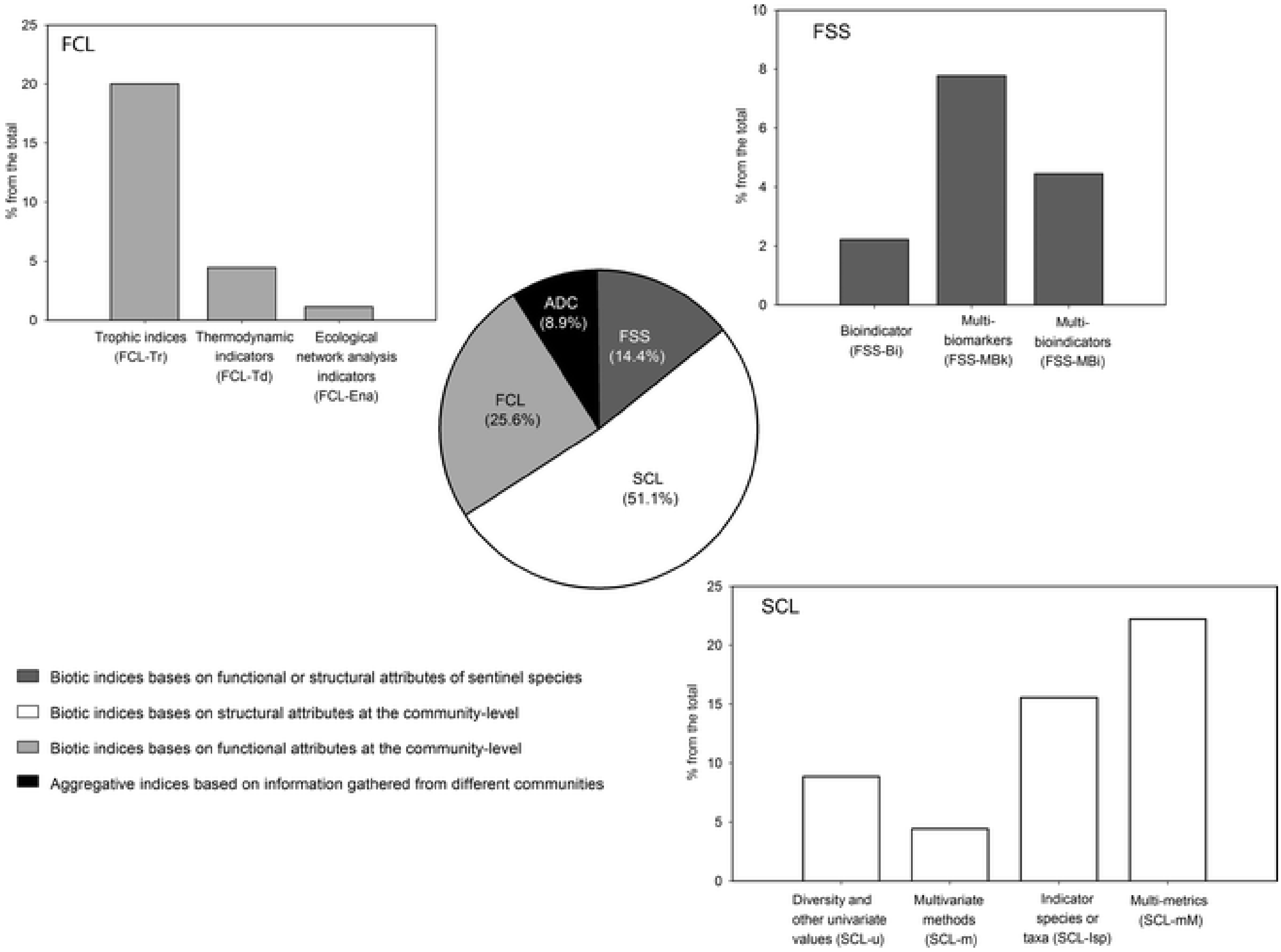


Fig. 3 Relative distribution of the 90 reviewed biotic indices (as %) between the four types proposed (FSS, SCL, FCL and ADC inside the circle), and when these are present, between groups within each type (bar-graphs).

- Biotic indices based on functional and/or structural attributes of sentinel species (FSS, 14.4%).
- Biotic indices based on structural attributes at the community-level (SCL, 51.1%).
- Biotic indices based on functional attributes at the community-level (FCL, 25.6%).
- Aggregative indices based on information gathered from different communities (ADC, 8.9%).

In the following sections, we define each one of these types, and analyze within-type variability providing adequate examples (see Table 1 and Fig. 3).

Biotic indices based on functional and/or structural attributes of sentinel species

Sentinel species are usually selected for practical (*e.g.* ease of culture, well-known biology), ecological (*e.g.* species occupying critical trophic positions, especially sensitive, ecosystem engineers[20]), or occasionally economic reasons (*e.g.* species of economic relevance). These are expected to provide mechanistic alerts for other components of the ecosystem (ref. 21, but see also ref. 13). Single bioindicators based on structural attributes at the supra-individual level of a sentinel species (FSS-Bi) have been used successfully in water quality assessments. However, the combination of multiple functional and/or structural attributes of one, or less frequently more than one, sentinel species is a more common approach. Among them, the concurrent assessment of several biomarkers (FSS-MBk), for which the attributes are variables at sub-individual level, is widespread. This approach takes advantage of the fact that the bulk of published papers in the field of bioindicators address the response of sentinel species to specific pollutants at the molecular, biochemical, genetic, cellular, immunological and physiological level (Fig. 4). These multi-biomarker indices have been developed for mussels, fishes, amphipods and sea urchins. The use of a combination of several biomarkers obtained from different sentinel species together with the measure of the study region's most relevant contaminants found in the organisms has also been proposed (see Table 1).

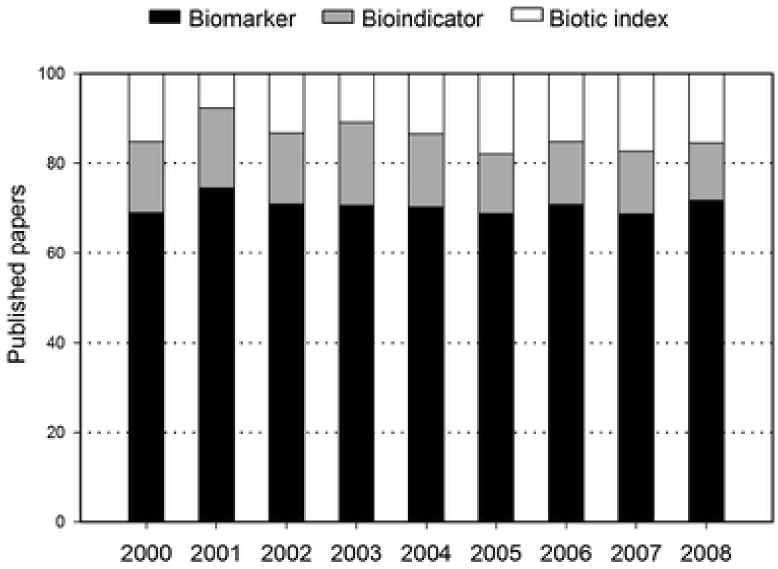


Fig. 4 Papers focused on the topic of biomarkers, bioindicators and biotic indices published from 2000 to 2008 in 96 ecological, toxicological and environmental science journals. Search performed on the ISI Web of Knowledge database.

Multi-bioindicator indices (FSS-MBi) are wider in scope, as they combine bioindicators obtained from levels ranging from biochemical to community and, therefore, bioindicators with different timings of response and different specificity to stressors[22] (see also Fig. 2). These indices have been mainly developed in fishes, molluscs, and seagrasses (see Table 1).

Biotic indices based on structural attributes at the community-level

The sensitivity to environmental changes of the biotic assemblage's taxonomic composition is widely recognized, and biotic indices based on this aspect are frequent in the literature (Fig. 3). However, the targeted taxonomic group of species usually only encompasses a part of the whole organism assemblage, typically a macrotaxon or syntaxon. The most commonly used groups for this type of indices are benthic macroinvertebrates (35%) and phytoplankton (18%). Most of them (93%) require taxonomic identification to either species level or the lowest possible level, whereas others (7%) adhere to the principle of taxonomic sufficiency, and require a low taxonomic resolution, such as identification only to the level of zoological groups (see Table 1).

Using this broad approach, different specific strategies have been applied. A first approach includes indices based on diversity values or other univariate expressions derived from the specific composition (SCL-u). For example, univariate measures based on the number of species (species richness, Margalef index), on species dominance or abundance distribution (Shannon-Wiener index, Menhinick's index, Evenness index), or on the taxonomic separation between each pair of species (taxonomic distinctness index) have been successfully applied to determine the status of phytoplankton, benthic macroinvertebrates and fishes. A second approach uses multivariate techniques to extract information about status from the matrix of species–samples, either qualitative or using adequate expressions of abundance (SCL-m). These indices have been developed for the epiphytic community of seagrass leaves and for rocky-shore, macroinvertebrate and fish communities. A third approach is based on the measure of the presence, biomass or abundance of indicator species or taxa of known sensitivity or tolerance to disturbances (SCL-Isp). This approach has been successfully applied on phytoplankton, macroalgae, seagrasses and macroinvertebrates. Generally, these indices are based on assigning a weight to sensitive/tolerant species. A last approach integrates and combines different taxonomical measures into a single score for assessing the ‘biotic integrity’ (SCL-mM including IBIs). The combination of individual metrics, scored according to their respective references, or to their mean and standard deviation in the test dataset, is performed by averaging, using a linear combination, or using statistical multivariate methods. The principles of the IBI were first developed in freshwater systems, and more recently they have been used in coastal waters to assess the integrity of fish, benthic macroinvertebrate, phyto- or zoo-plankton, seagrass, and macroalgal communities (see Table 1).

Biotic indices based on functional attributes at the community-level

Biotic indices in this group are based on the assumption that, in addition to altering species functioning and taxonomic composition, human impact also affects the energy transfer between trophic levels and species interaction, or, more generally, ecosystem functioning. Under this broad notion, two approaches have been attempted: one focusing on trophic aspects, and the other on holistic expressions of ecosystem condition derived from ecological theory.[23]

Within the trophic approach (FCL-Tr) several options have been used. The oligotrophy/eutrophy of aquatic ecosystems has been assessed by measuring the biomass or photosynthetic parameters of primary producers. Some of these measures (mainly based on chlorophyll-a) have been adapted to assess the ecological status of coastal waters. Other indices based on classifying species into functional groups (*e.g.* feeding groups, morphological groups) have been developed for fish, benthic macroinvertebrate, macroalgal, and plankton communities. Trophic indices have also been combined with other approaches, for example, chemical measures or taxonomic information. Finally, we include here indices based on integrative measures such as the estimation of metabolic rates (*i.e.* oxygen exchange), or the estimation of body-size or size spectra (see Table 1). The size-based indices are included here because of the underlying assumption of a positive correlation between body mass and trophic level,[24] together with the consideration that increasing organic pollution results in the loss of the larger long-lived species (*k*-strategists) from the community in favour of the smaller and more tolerant short-lived opportunistic species (*r*-strategists).[25]

On the other hand, ecosystem theory seems to offer two main ways for assessing the ecosystem status:[26] one is based on ecological network analysis (FCL-Ena), while the other uses thermodynamic concepts (FCL-Td). Some properties based on network analysis have been proposed (emergy, ascendancy), but, to our knowledge, they have not yet been applied. The two most broadly used indicators in this group are derived from the field of thermodynamics: eco-exergy and specific exergy. Eco-exergy is defined as the chemical energy embodied in an ecosystem's organic compounds and biological structures, and measures the distance from thermodynamic equilibrium of a system which stores biomass and information in the form of coding genes.[27,19] Specific eco-exergy is the exergy normalized to biomass. Although it is not possible to calculate these two properties for the entire ecosystem,[28] they have been successfully applied to certain macrotaxa or subsets of organisms in integrity assessments of estuaries, harbours, and coastal lagoons (see Table 1), and in a rocky shore community recovery experiment.[29]

Aggregative indices based on information gathered from different communities

These indices are based on the aggregation of multiple biotic indices of the previous types obtained from different communities. Tentatively, such indices have been calculated as the weighted sum, the average of the partial components, or by using multivariate ordination and ranking methods (see Table 1). These aggregative or composite indices apply the underlying concepts of IBIs, and are dependent on a suitable selection of the individual indices, on their division into categories of condition, on a region-specific definition of reference conditions for the different indices included,[30] and on the method used to aggregate the individual index values (*e.g.* weighting schemes).

Table 1 Details and classification of the biotic indices reviewed. The revision used the search of indices performed on the ISI Web of Knowledge database (see Fig. 1) as starting point, being enlarged to previous years in order to include the references that first cited and successfully applied them in the context of the large-scale strategies (highlighted in bold). Asterisk (*) denotes identification to species level or lowest possible level

Index category	Index type	Biotic index (short-name)	Target community/ species	Taxonomic resolution	Habitat	Geographic regions of development (reference in black) or successful application	Reference conditions
Indices based on functional or structural attributes of sentinel species (FSS)	*Single bioindicator at supra-individual level* (*FSS-Bi*)	Conservation index (CI)	Seagrasses (*Posidonia oceanica*)	Monospecific	Marine	NW Mediterranean (**31**,32,33)	Available data and expert judgment
		Depth limit	Seagrasses (*Zostera marina*)	Monospecific	Marine	Baltic Sea (**34**,35)	Historic data
	Multi-biomarker indices (*FSS-MBk*)	Animal health index (Expert System 6,0 software)	Mussels	Monospecific	Marine	Baltic Sea (**36**), North Sea (37)	Virtual reference conditions
		Cumulative toxicity index	Amphipod (*Ampelisca abdita*) and sea urchin (*Arbacia punctulata*)	Bispecific	Estuarine	NW Atlantic (**38**)	Non-defined
		Immunotoxicological index (no name)	Mussels (*Mytilus galloprovincialis*)	Monospecific	Marine	W Mediterranean (**39**)	Non-defined
		Multimarker pollution index (MPI)	Mussels	Monospecific	Marine	Mediterranean (**40**)	Minimally disturbed reference sites

		Multivariate analysis of biomarker responses (no name)	Mussels (*Mytilus edulis*) and crabs (*Carcinus maenas*)	Bispecific	Estuarine	NE Atlantic (**41**)	Minimally disturbed reference sites
		Rapid assessment of marine pollution (RAMP)[a]	Several species	—	Marine/ estuarine/ lagoon	None (**42**)	Non-defined
		Integrated biomarker index (IBR)	Fishes and mussels	Monospecific	Marine/ estuarine	NE Atlantic (**43**), Baltic Sea (modified by 44–46), NW Mediterranean (47)	Non-defined
	Multi-bioindicator indices (*FSS-MBi*)	*P. oceanica* multivariate index (POMI)[e]	Seagrass (*P. oceanica*)	Monospecific	Marine	NW Mediterranean (**48**)	Virtual reference sites and expert judgment
		Multiple indicators for fish communities (no name)[e]	Fishes	Functional groups	Marine	NW Atlantic (**49**)	Non-defined
		Health status of *Mya arenaria* (no name)	Bivalve (*M. arenaria*)	Monospecific	Estuarine	NW Atlantic and Baltic Sea (**50**)	For each individual metric
		Bioeffect assessment index (BAI)[d]	Fishes (*Platichthys flesus*, *Zoarces viviparus*)/mussels (*Mytilus* spp.)	Mono- or multi-specific	Marine	North Sea (51), Baltic Sea (44)	Baseline levels in unimpaired organisms
Biotic indices based on structural attributes at the community-level (SCL)	*Diversity and other univariate indices* (*SCL-u*)	Species richness (S)	Phytoplankton	Species*	Marine	E Mediterranean (52)	Minimally disturbed reference sites
		Margalef index (I)	Macroinvertebrates	Species*	Marine/ estuarine/ lagoon	NW Mediterranean (53 for amphipods), NE Atlantic (54), NW Mediterranean (55)	Available data (56)
		Shannon-Wiener (H')	Phytoplankton	Species*	Lagoon	Black Sea (57)	Non-defined
			Macroinvertebrates	Species*	Marine	NW Mediterranean (53 for amphipods, 58)	Available data (56)
		Evenness index (E)	Phytoplankton	Species*	Marine	E Mediterranean (52)	Minimally disturbed reference sites
		Menhinick's index (DMn)	Phytoplankton	Species*	Marine	E Mediterranean (52)	Minimally disturbed reference sites

	Kothe's species deficit (Dk)	Phytoplankton	Species*	Marine	E Mediterranean (52)	Minimally disturbed reference sites
	Pielou eveness index	Phytoplankton	Species*	Lagoons	Black Sea (57)	Non-defined
	Taxonomic distinctness index (Δ)	Macroinvertebrates	Species*	Marine/ estuarine	North Sea (**59**), NE Atlantic and SE Pacific (60,61)	Reference master list of taxa (55)
		Fishes	Species*	Marine	NE Atlantic and North Sea (62)	Non-defined
Multivariate indices (*SCL-m*)	Epiphytic community of seagrass leaves (no name)	Seagrass epiphytes	Species*/ taxonomic groups	Marine	NW Mediterranean (63)	Non-defined
	Composition and structure of rocky-shore communities (no name)	Rocky-shore communities	Species*	Marine	NW Mediterranean (64)	Non-defined
	Macroinvertebrates of soft-bottom benthos (no name)	Macroinvertebrates	Species*	Estuarine/ harbors	SE Pacific (65)	Minimally disturbed reference sites
	Community degradation (or disturbance) index (CDI)	Fishes	Species*	Estuarine/ lagoon	SW Indian Ocean (**66**,67)	Historical data and expert judgment
		Macroinvertebrates	Species*	Marine	North Sea (68)	Minimally disturbed reference samples
Indicator species or taxa (*SCL-Isp*)	Abundance or blooms of indicator species	Phytoplankton	Species*	Marine	Cantabrian Sea (69), NE Atlantic (70), Baltic Sea (35)	Available data and expert judgment (70)
	Substitution index (SI)	Seagrasses	Species	Marine	E Ligurian Sea (**32**,33)	Available data and expert judgment (32)
	Rapid-macrophyte quality index (R-MaQI)	Macroalgae and seagrasses	Species*	Lagoon/ estuarine	NW Mediterranean (**71**)	Expert judgment
	Macroalgal blooms	Macroalgae	Species*	Intertidal	NE Atlantic (72)	Available data and expert judgment
	Upstream fucoid penetration-limit	Macroalgae	Taxonomic group (fucoid identification)	Estuarine	NE Atlantic (**73**)	Minimally disturbed reference sites
	Cartography of littoral and upper-sublittoral rocky-shore communities (CARLIT)[b]	Macroalgae, seagrasses and *Mytilus*	Species*, genus and taxonomic groups	Marine	NW Mediterranean (**74**)	Minimally disturbed reference sites

	Azti marine biotic index (AMBI), initially named biotic coefficient (BC)[b]	Macroinvertebrates	Species*	Marine/ estuarine/ lagoon	Cantabrian Sea (**75**), NE Atlantic and Mediterranean (76,77,55)	Virtual reference conditions and expert judgment
	Benthic index (BENTIX)[b]	Macroinvertebrates	Species*	Marine/ lagoon	Mediterranean Sea (**78**,79), NE Atlantic (77)	Minimally disturbed reference sites (78)
	Benthic quality index (BQI)[b]	Macroinvertebrates	Species*	Marine	Baltic Sea (**80**), NE Atlantic (77), NW Mediterranean (58)	Virtual reference conditions and expert judgment (80)
	Benthic response index (BRI)[b] (using ordination methods)	Macroinvertebrates	Species*	Marine	Mid-W Pacific (**81**)	Minimally disturbed reference samples
	Macrofauna monitoring index (no name)[b]	Macroinvertebrates	Species*	Marine	SW Pacific (**82**)	Minimally disturbed reference sites
	Benthic opportunistic polychaetes/ amphipods (BOPA) index[b]	Macroinvertebrates	Zoological groups	Marine/ estuarine	NE Atlantic (**83**,77,84)	Available data and expert judgment (84)
	Benthic Opportunistic Annelida/Amphipods (BO2A) index[b]	Macroinvertebrates	Zoological groups	Estuarine	NE Atlantic (**85**)	Non-defined
	Relative benthic index (RBI)	Macroinvertebrates	Species*	Estuarine	California Bay (86)	Available data and expert judgment
Multi-metric indices (*SCL-mM*)	Synthetic maps (no name)	Phytoplankton	Species*	Marine	NE Mediterranean (87)	Available data and expert judgment
	Phytoplankton index of biotic integrity (P-IBI)[a,c]	Phytoplankton	Species*/ taxonomic groups	Estuarine	Chesapeake Bay (**88**)	Minimally disturbed reference samples
	IBI based on the summer polyhaline zooplankton	Zooplankton	Species*/ taxonomic groups	Estuarine	Chesapeake Bay (89)	Minimally disturbed reference samples
	Macroalgae composition and coverage (no name)[d]	Macroalgae and seagrasses	Species*/ ecological groups	Marine/ estuarine	Cantabrian Sea (69)	Available data and expert judgment
	Quality of rocky bottoms (CRF)[d]	Macroalgae	Species*	Marine	Cantabrian Sea (**90**)	Available data and expert judgment

Phase-shift index (PSI)[d]	Seagrasses and macroalgae	Species	Marine	E Ligurian Sea (33)	Available data and expert judgment
Seagrass composition and abundance (no name)[c]	Seagrasses	Species*	Marine	NE Atlantic (91)	Available data and expert judgment
Estuarine index of biotic integrity (IBI)	Macroinvertebrates and submerged aquatic vegetation	Species*/ taxonomic groups	Estuarine	NW Atlantic (92)	Minimally disturbed reference sites
Macrobenthic index in sheltered systems (MISS)[c]	Macroinvertebrates	Species*/ ecological groups	Lagoon	NE Atlantic (**93**)	Minimally disturbed reference sites
Ecological quality status (no name)[a,c]	Macroinvertebrates	Species*/ ecological groups	Marine/ estuarine	NE Atlantic (**77**)	Reference conditions of the different metrics integrated
TICOR approach[c]	Macroinvertebrates	Species*/ ecological groups	Estuarine	NE Atlantic (94)	Available data and expert judgment
EMAP-Virginian Province benthic index (BI)[a]	Macroinvertebrates	Species*/ zoological groups	Estuarine	NW Atlantic (95,**96**)	Minimally disturbed reference sites
Benthic index of biotic integrity (B-IBI)[a,c]	Macroinvertebrates	Species*/ ecological groups	Estuarine	NW Atlantic (**97**, modified by 98,99)	Minimally disturbed reference sites (97) and degraded sites (99)
Estuarine benthic index of biotic integrity (B-IBI) for Mid-Atlantic integrated assessment program (MAIA)[a,c]	Macroinvertebrates	Species*/ ecological groups	Estuarine	Mid-W (**100**) and NW (101) Atlantic	Minimally disturbed reference sites
Benthic index of environmental condition (no name)[a,d]	Macroinvertebrates	Species*/ ecological groups	Estuarine	Mid-W (**102**) and NW (modified by 103) Atlantic	Minimally disturbed reference sites
Tampa Bay benthic index (TBBI)[a,d]	Macroinvertebrates	Species*/ ecological groups	Estuarine	NW Atlantic (**104**)	Available data and expert judgment
Multivariate-AMBI (M-AMBI)[e]	Macroinvertebrates	Species*	Marine/ estuarine	Cantabrian Sea (**105**)	Virtual reference conditions and expert judgment

		Estuarine biotic integrity index (EBI)[c]	Fishes	Species*/ ecological groups	Estuarine	NW (**106**, 109) and N (modified by 108) Atlantic; North Sea (modified by 107)	Non-defined
		Fish recruitment index (FRI)[a]	Fishes	Species*	Estuarine	SW Indian Ocean (110)	Non-defined
		Estuarine fishes (no name)[d]	Fishes	Species*/ ecological groups	Estuarine	North Sea (14); Cantabrian Sea (69)	Available data and expert judgment
Biotic indices based on functional attributes at the community-level (FCL)	*Trophic indices* (*FCL-Tr*)	Biomass (total cell number or chlorophyll-a concentrations)[f]	Phytoplankton	None	Marine	E (**52**) and NE (modified by 112) Mediterranean, Cantabrian Sea (modified by 69), NE Atlantic (modified by 70 and 111)	Available data and expert judgment
		Trophic status index (TSI)[f]	Phytoplankton/ macrophyte	Species*	Lagoon	NE Pacific (113)	Non-defined
		Synthetic trophic index (*I*)[f]	Phytoplankton	None	Marine/ estuarine/ lagoon	SW Indian Ocean and SW Mediterranean (114)	Non-defined
		Benthic trophic status index (BTSI)[a,g]	Photoautotrophy *versus* heterotrophy (oxygen exchange)	None	Estuarine	NW Atlantic (115)	Non-defined
		Index of size distribution (ISD)[g]	Macroinvertebrates (estimation of body-size or size spectra)	None	Lagoon	NE Mediterranean (116)	Minimally disturbed reference sites
		Trophic Index (TRIX)[a]	Phytoplankton	None	Marine/ lagoon	NW Adriatic Sea (**117**), Adriatic and Tyrrhenian Sea (118), Black Sea (57)	Non-defined
		Unscaled TRIX (UNTRIX)[a]	Phytoplankton	None	Marine	NW Mediterranean (119)	Minimally disturbed type-specific reference sites
		Seasonal succession of functional groups	Phytoplankton	Functional groups	Marine	North Sea (120), Baltic Sea (121), NE Atlantic (modified by 70)	Available data and expert judgment
		Photopigments[h]	Phytoplankton	Functional groups	Marine/ estuarine	Mid-Atlantic (122)	Non-defined

		Ecological evaluation index (EEI)[h]	Macroalgae	Genus	Marine	NE Mediterranean (**123**,**124**,125)	Minimally disturbed reference sites (125)
		Macroalgal composition tool (and Reduced Species List, RSL, tool)[i]	Macroalgae	Species*/ functional groups	Marine	NE Atlantic (126)	Available data and expert judgment
		Infaunal trophic index (ITI)[h]	Macroinvertebrates	Species*/ functional groups	Marine/ estuarine	Mid-E Pacific (**127**); NE Atlantic (77)	Available data and expert judgment
		Mean trophic level (TLm)[h]	Fishes	Species*/ functional groups	Marine	NW and NE Atlantic, SE Pacific, Mediterranean (**128**,**129**); SW Atlantic (130)	Non-defined
		Fishing-in-balance index (FIB)[h]	Fishes	Species*/ functional groups	Marine	NW (**131**) and SW (130) Atlantic	Non-defined
		Biomass trophic level spectra (BTLS)[h]	Fishes	Species*/ functional groups	Lagoon	Mid-W Atlantic (132)	Non-defined
		Estuarine fish community index (EFCI)[d,i]	Fishes	Species*/ functional groups	Estuarine	SW Indian Ocean (133)	Best values of the metrics on the dataset
		Transitional fish classification index (TFCI)[i]	Fishes	Species*/ functional groups	Estuarine	North Sea (134)	Non-defined
		Conservation priority index for estuarine fishes (COPIEF)	Fishes	Species*/ functional groups	Estuarine	NE Atlantic (**135**)	Expert judgment
	Thermodynamic indicators (*FCL-Td*)	Ecosystem exergy storage (Eco-exergy)	Several communities		Estuarine/ lagoon/ harbour	NE Atlantic (54), E Pacific (136), Mediterranean (137)	—
		Specific exergy storage (SpEx)	Macroalgae and seagrasses		Estuarine/ lagoon	NE Atlantic (54), Mediterranean (137)	—
		Buffer capacity	—	—	—	None (138)	—
		Specific entropy production	—	—	—	None (139)	—
	Ecological network analysis indicators (*FCL-Ena*)	Ascendancy	—	—	—	None (26,140)	—
Aggregative indices based on information gathered from different communities (ADC)		Basque integrated assessment (no name)[a,i]	Several communities	For each individual component	Marine/ estuarine	Cantabrian Sea (69,141)	For each individual component
		Integrated classification (no name)[i]	Several communities	For each individual component	Marine	NE Mediterranean (112)	For each individual component

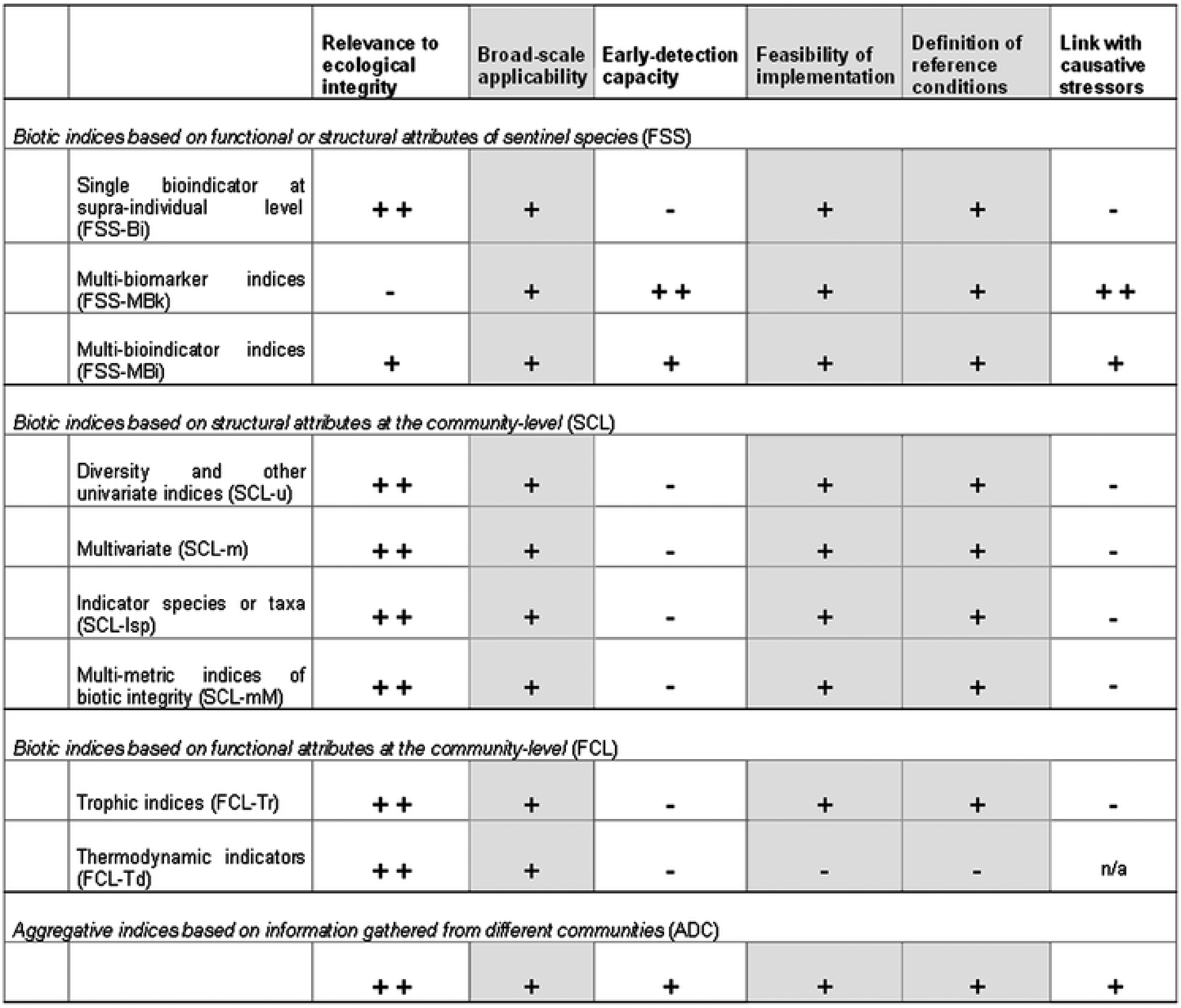
Index of ecosystem integrity (no name)[a,e]	Several communities	For each individual component	Estuarine	NW Atlantic (142)	For each individual component
Estuarine QUAlity and condiTION (EQUATION)[a,j]	Several communities	For each individual component	Estuarine	Mid E Pacific, North Sea, NE Atlantic (143)	For each individual component
Ecofunctional quality index (EQI)[d]	Several communities	For each individual component	Lagoon	Adriatic Sea (144)	For each individual component
Assessment of estuarine trophic status (ASSETS)[a]	Several communities	For each individual component	Estuarine	Mid and NW Atlantic (145)	—
Index of environmental integrity (IEI)[c]	Several communities	For each individual component	Estuarine	Mid-W Atlantic (146)	For each individual component
Bay health index (BHI)[a,c]	Several communities	For each individual component	Estuarine	NW Atlantic (**30**)	For each individual component

[a] Combined with chemical measures. [b] Weighting sensitive/tolerant species. [c] Combination of individual metrics or partial components by averaging. [d] Combination of individual metrics or partial components by using a linear combination or sum. [e] Combination of individual metrics or partial components by using statistical multivariate methods. [f] Measuring the biomass or photosynthetic parameters of primary producers. [g] Based on integrative measures. [h] Based on classifying species into functional groups. [i] Combined with taxonomic information. [j] Partial components combined using decision support system (usually the worst status of the biological elements).

Strengths and weaknesses linked to each type of indices

Finding the "perfect index", understood as that fulfilling all the requirements outlined above, is still a scientific challenge. Each index, and broadly each type of indices, has their specific strengths and weaknesses, which we explore and summarize below (see also Table 2).

Table 2 Strengths (+ +) and weaknesses (–), in relation to the requirements defined in the text, of the four types of indices and of the groups within each type (short names between brackets). An intermediate rating (+) denotes a strength or weakness depending on how the index is applied. Aspects that show problems common to all index types are marked in grey. N/a: not available



		Relevance to ecological integrity	Broad-scale applicability	Early-detection capacity	Feasibility of implementation	Definition of reference conditions	Link with causative stressors
Biotic indices based on functional or structural attributes of sentinel species (FSS)							
	Single bioindicator at supra-individual level (FSS-Bi)	+ +	+	-	+	+	-
	Multi-biomarker indices (FSS-MBk)	-	+	+ +	+	+	+ +
	Multi-bioindicator indices (FSS-MBi)	+	+	+	+	+	+
Biotic indices based on structural attributes at the community-level (SCL)							
	Diversity and other univariate indices (SCL-u)	+ +	+	-	+	+	-
	Multivariate (SCL-m)	+ +	+	-	+	+	-
	Indicator species or taxa (SCL-Isp)	+ +	+	-	+	+	-
	Multi-metric indices of biotic integrity (SCL-mM)	+ +	+	-	+	+	-
Biotic indices based on functional attributes at the community-level (FCL)							
	Trophic indices (FCL-Tr)	+ +	+	-	+	+	-
	Thermodynamic indicators (FCL-Td)	+ +	+	-	-	-	n/a
Aggregative indices based on information gathered from different communities (ADC)							
		+ +	+	+	+	+	+

Biotic indices based on functional and/or structural attributes of sentinel species

Indices based on a single bioindicator at the supra-individual level are usually easy to measure and interpret. Moreover, they usually reflect overall ecosystem integrity since the chosen species are expected to play an important ecological role. However, as those indices are commonly based on some kind of appreciation of species abundance, they are limited as regards two aspects. On the one hand, they fail in the early detection of disturbances, as abundance decrease takes place in an advanced stage of deterioration. On the other hand, they do not provide information on causative stressors, as such abundance decrease is unspecific. By contrast, multi-biomarker indices allow the early detection of disturbances as they respond at largely sub-lethal impacts, and can help to link biological degradation to its causative stressors when combined with additional information (*e.g.* chemical data, pollution sources, toxicity tests).[147] However, as they are based on sub-individual levels, they do not adequately reflect overall ecosystem integrity. In effect, the links between changes in biomarkers and the effects on the health or fitness of individual organisms, and the effects on populations, communities or ecosystems are difficult to establish.[148] Of course, changes at the sub-individual level can ultimately propagate towards the individual (*e.g.* fitness, reproduction, growth), and population or community levels, but only when a certain threshold of the pollutant has been reached or when the internal compensatory mechanisms have been exceeded.[149,150] In other words, such stress propagation is non-linear, resulting in an unclear relationship between biomarkers and ecosystem integrity. Multi-bioindicator indices encompass diverse levels of biological organization, and are expected to provide a more complete overview of ecosystem integrity, while improve the understanding of the interactive effect of multiple stressors, both sub-lethal (early-warning) and lethal (for the latter response).[151,152] However, that is only true when they combine biomarkers with community-level indicators,[48,51] which concomitantly increase the cost and technical difficulties. When multi-bioindicator indices do not incorporate indicators of population or community levels,[44,50] they are not relevant to the ecological integrity. By contrast, when they do not incorporate biomarkers[49] fail in the early-detection of disturbances and in providing information on the causative stressors. Moreover, indices based on both a single bioindicator at the supra-individual level and on the combination of multiple biomarkers and/or bioindicators continue to present shortcomings, especially related to their applicability on a large spatial scale:

(i) they depend on the geographic distribution of one sentinel species, often widely distributed but not ubiquitous;[46]

(ii) they vary widely, depending on natural biological and environmental factors such as small-scale heterogeneity,[153] seasonal and interannual variability,[154,155] and the times required for induction, adaptation and recovery of biological responses.[156] In the case of biomarkers, the tissue analysed, and the sex, age, nutritional and reproductive status of the organism, among others, should be added to the list;

(iii) they do not always respond in a simple, linear and predictable way to anthropogenic disturbances, independently of the spatial scale considered.

Consequently, the development of multi-biomarker and multi-bioindicator indices requires a careful process of selection, validation and aggregation of individual indicators. This process should be aimed at maximizing the index ability to discriminate among different degrees of deterioration over large spatial-scales and minimizing natural variability.[21,157] However, this is not always performed, since single bioindicators are mainly developed on a local scale or under controlled laboratory conditions, assessing the response elicited by a single stressor. Moreover, when this process has been performed from an array of candidate indicators believed or known to react to disturbances (at least on some spatial scale), only about 30% of them properly detect the deterioration gradients.[49,157] This statistic illustrates the relatively frequent mismatch that occurs between the scale on which single bioindicators are generally developed, and that on which the multi-metric indices based on them, and ecosystem management decisions must be implemented.

Biotic indices based on structural attributes at the community-level

The main advantage of these indices is their ability to reflect the overall ecosystem condition. Moreover, their response to the combined effects of multiple stressors comes close to that of the multi-bioindicator indices in some cases.[63] When using univariate diversity indices, caution is recommended because they are highly dependent on natural factors (*e.g.* seasonal variability, habitat type, massive recruitment events or patchy distribution of species)[158] or methodological considerations (*e.g.* sampling size, sampling methodology, appropriate selection of sites representing extreme conditions for index validation, criteria used to define the reference conditions).[159–161] By contrast, biotic indices based on weight assignment to groups of tolerant or sensitive species represent a promising approach which avoids the problems resulting from seasonal variability of communities.[159] However, when these indices have been tested over a large range of geographical areas, other problems and inconsistencies have been identified.[56,79,158,162] These are due not only to biological and environmental variability, but also to the fact that most species and taxa are not present at all the sites being compared, and also to the assignation of certain species to unsuitable or erroneous groups. This has led to the development of extended families of analogous region-specific indices that differ only subtly from one another.[15,163] The classification of species into different categories or ecological groups, and the weight coefficients assigned to them are often more or less subjective aspects, in which the experience and expertise of the scientist play a significant role.[164,165]

The main weaknesses common to indices in this category include a lack of specificity to stressors,[166] and the general failure to detect deterioration at an early stage, as some effect at the supra-individual level (*e.g.* species abundance increase or decrease) must usually take place in order to alter the index value. Additionally, the use of taxonomy-based indices is constrained by their dependence on an adequate level of taxonomic expertise which should be constantly updated. Most taxonomy-based indices require taxonomic identification to species level, and errors in species identification could lead to incorrect classifications and misinterpretations of the data, eventually discrediting both ecological studies and biotic indices.[167]

Biotic indices based on functional attributes at the community-level

These indices are obviously thought to provide a fairly accurate picture of ecosystem integrity, and they are believed to respond in an integrative way to multiple stressors. Currently, however, the feasibility of implementing such indices remains limited. In fact, trophic indices are usually applied to restricted subsets of organisms (syntaxa: phytoplankton; taxa: fishes, see Table 1). To properly trace the flux of matter and energy through the system in a holistic manner, more trophic levels (as a minimum, primary producers, microbial, herbivores and secondary consumers), and more types of organisms representing each level should be incorporated, including groups of special relevance in the carbon flux.[168] Moreover, there are significant complications regarding applicability of such indices, as trophic divisions are often diffuse due to changes in diet or feeding intensity caused by seasonality, life cycle, species distribution or habitat diversity.[16,169]

In the case of thermodynamic indices, the genetic parameters required are difficult to quantify,[170] and the indicators' dependence on the organisms' biomass can lead to inconsistent results and seasonal fluctuations.[54] Additionally, only contributions from major components of biomass and genetic information, and not for the entire ecosystem, are usually taken into account for the calculation of eco-exergy. Consequently, the application of these indicators to the assessment of ecosystem health is only of interest when comparing the exergy differences between two different structures.[28] In their current state, thermodynamics indices show practical and conceptual difficulties, although perhaps in the future, helped by the rapid developments in genetic tools and knowledge, they will be able to offer a novel and more complete picture.

On the other hand, trophic and thermodynamic indices do not link ecosystem degradation to the causative stressors. Specially, thermodynamic indices need to be tested under a wider range of stressors and conditions to be useful in the implementation of large-scale water quality monitoring programs.

Aggregative indices based on information gathered from different communities

Due to the fact that these indices combine indices from the previous types obtained from different communities, they provide a fairly integrative approach. Moreover, as these different communities respond differently to disturbances, they allow a better interpretation of the interactive effects of multiple stressors, and improve our understanding of their ecological consequences. However, they usually integrate available and not necessarily complementary individual indices (*e.g.* for SCL-mM),[30] and are also subjected to some constraints. These constraints are the result of the individual index weaknesses already outlined in the previous sections. Moreover, the optimum means of aggregating these indices into a single value remains unsolved,[141] curtailing the immediacy of their interpretation. Additionally, the need for several indices based on different communities increases economic cost as well as technical difficulties. At the same time, it implies a strongly coordinated effort, as different research groups working in unison are generally required in order to attain the necessary scientific expertise.

Lessons learned and future perspectives

Research into basic and applied issues related to bioindicators has primarily been carried out in the past 40 years.[171] Substantial efforts have been made to transfer this knowledge to society, with significant benefits to management, ecosystems protection and, ultimately, human welfare.[147] However, the needs identified here are still far from being completely met by any existing index (see Table 2).

On the one hand, there are certain problems common to all index types, including large-scale applicability, and the definition of adequate reference conditions. There is an increasing tendency to plan coastal water management over large geographical areas. Consequently bioindicators should be obtained from widely distributed species or communities, and should show robustness to geographical variability. This robustness is difficult to achieve, as it faces both bio-ecological problems (*e.g.* a species can behave differently in different areas of its geographical range) and methodological shortcomings (*e.g.* methods optimized to accommodate local constraints can be inadequate when they are transferred to another area). It is strongly recommended that any new method of ecological status assessment should be designed and validated for large-scale application, and adapted to different areas.[172] This is especially true when indices developed in coastal waters are transferred to transitional waters and semi-enclosed coastal systems[173,174] or *vice versa*.[169] Concerning reference conditions, it should be stressed that the different criteria used for their definition have an important effect on the precision and robustness of biotic indices, and therefore on the final assessment of the aquatic ecosystem's status. Consequently, there is a need for a solid consensus, especially in regions where it is not possible to find pristine or near-pristine zones. This consensus should be based not only on the continued implementation of long-term and extensive monitoring programs, on intercalibration exercises,[175–177] and on the definition of confidence limits to account for natural variability in reference conditions,[56,99,160] but also on further research aimed at understanding the real structure and functioning of pristine ecosystems and the base-line shift.

On the other hand, some degree of incompatibility appears between two basic groups of properties. Relevance to ecological integrity seems opposed to specificity to individual stressors and early-detection ability. Most biotic indices based on structural or functional attributes at the community-level and on a single bioindicator at the supra-individual level provide an integrative view of the ecosystem status; however, they usually lack specificity and have a poor early-detection capacity. Conversely, the opposite is true for multi-biomarker indices. Of course there are means to partially solve this antithesis. For example, the lack of early detection ability shown by biotic indices based on structural attributes at the community level can be solved by assessing highly sensitive communities that respond to stressors faster than others (*e.g.* phytoplankton). Similarly, their lack of specificity can be solved by the concurrent measure of more specific bioindicators of stress (*e.g.* biomarkers).[178–180] However, this will pose problems of feasibility, including increasing cost and complexity in the index construction. In contrast, all three aspects are to some extent covered by multi-bioindicator indices that include different organization levels, and, in most cases, aggregative indices.

The research for the "perfect index" seems to some extent a kind of scientific quest for the Holy Grail. More research is still needed, not only for a better understanding of the bioindicators' behavior, but also for linking this behavior to structural and functional aspects of the ecosystem. Moreover, an effort should be made to better frame the field of bioindicators within the general ecological theory. Finally, it should be always taken into consideration that the field of bioindicators is at the interface between science and management, and, therefore, society. Scientists in this field should be especially sensitive to communication needs, and provide tools with acceptable levels of understandability and interpretability.

We conclude that not only does a "perfect biotic index" not exist, but also that there is no "optimum biotic index", *i.e.* a single index performing better than all the others with regard to all of the six aspects examined in this review. Consequently, and given the present state of knowledge, the best strategy for an optimum assessment of the ecological status of coastal waters is the simultaneous use (and aggregation) of several indices with complementary strengths and obtained from different biological communities. Concerning the six aspects considered, the most adequate choices would be those which included SCL and FSS-Bi or FSS-MBk in the combination.

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