Handbook of Ecological Indicators for Assessment of Ecosystem Health. Jorgensen, S.E., Costanza, R.; Xu, F.-L. CRC Press. 2005

CHAPTER 2

Application of Indicators for the Assessment of Ecosystem Health

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This chapter provides a comprehensive overview of the wide spectrum of indicators applicable for the assessment of ecosystem health. The applied indicators are classified in seven levels: (1) application of specific species; (2) ratio between classes of organisms; (3) specific chemical compounds; (4) trophic levels; (5) rates; (6) composite indicators included E.P. Odum's attributes and various indices; (7) holistic indicators as, for instance, biodiversity and resistance; (8) thermodynamic indicator. The chapter shows by several examples (based on case studies) that the application of the seven levels are consistent, at least to a certain extent, i.e., that indicators in level 1 and 2, for instance, would give the same indication as indicators from for instance level 6 and 7. The chapter presents furthermore an ecosystem theory that is shown to be applicable as fundamental for the ecological indicators, particularly the indicators from level 6 and 7.

1-56670-665-3/05/$0.00+$1.50

2.1 CRITERIA FOR THE SELECTION OF ECOLOGICAL INDICATORS FOR EHA

Von Bertalanffy characterized the evolution of complex systems in terms of four major attributes:[1]

1. Progressive integration (which entails the development of integrative linkages between different species of biota and between biota, habitat, and climate).
2. Progressive differentiation (progressive specialization as systems evolve biotic diversity to take advantage of abilities to partition resources more finely and so forth).
3. Progressive mechanization (covers the growing number of feedbacks and regulation mechanisms).
4. Progressive centralization (which does probably not refer to a centralization in the political meaning, as ecosystems are characterized by short and fast feedbacks and decentralized control, but to the more and more developed cooperation among the organisms (the "Gaia" effect) and the growing adaptation to all other component in the ecosystem).

Costanza summarizes the concept definition of ecosystem health as:[2]

1. Homeostasis
2. Absence of disease
3. Diversity or complexity
4. Stability or resilience
5. Vigor or scope for growth
6. Balance between system components.

He emphasizes that it is necessary to consider all or least most of the definitions simultaneously. Consequently, he proposes an overall system health index, $HI = V \times O \times R$, where V is system vigor, O is the system organization index and R is the resilience index. With this proposal, Costanza touches on probably the most crucial ecosystem properties to cover ecosystem health.

Kay uses the term "ecosystem integrity" to refer to the ability of an ecosystem to maintain its organization.[3] Measures of integrity should therefore reflect the two aspects of the organizational state of an ecosystem: function and structure. Function refers to the overall activities of the ecosystem. Structure refers to the interconnection between the components of the system. Measures of function would indicate the amount of energy being captured by the system. Measures of structure would indicate the way in which exergy is moving through the system, therefore the exergy stored in the ecosystem could be a reasonable indicator of the structure.

Kay (1991) presents the fundamental hypothesis that ecosystems will organize themselves to maximize the degradation of the available work (exergy) in incoming energy[3] and that material flows will tend to close, which is necessary to ensure a continuous supply of material for the energy degrading processes. Maximum degradation of exergy is a consequence of the development of ecosystems from the early to the mature state, but as ecosystems cannot degrade more energy than that corresponding to the incoming solar radiation, maximum degradation may not be an appropriate goal function for *mature* ecosystems. This is discussed further in section 4 of this chapter. It should, however, be underlined here that the use of satellite images to indicate where an ecosystem may be found on a scale from an early to a mature system, is a very useful method to assess ecosystem integrity. These concepts have been applied by Akbari to analyze a nonagricultural and an agricultural ecosystem.[4] He found that the latter system, representing an ecosystem at an early stage, has a higher surface-canopy air temperature (less exergy is captured) and less biomass (less stored exergy) than the nonagricultural ecosystem, which represents the more mature ecosystem.

O'Connor and Dewling proposed five criteria to define a suitable index of ecosystem degradation, which we think can still be considered up-to-date.[5] The index should be:

1. Relevant
2. Simple and easily understood by laymen
3. Scientifically justifiable
4. Quantitative
5. Acceptable in terms of costs.

On the other hand, from a more scientific point of view, we may say that the characteristics defining a good ecological indicator are:

1. Ease of handling
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
5. Possible quantification.

It is not easy to fulfill all of these five requirements. In fact, despite the panoply of bio-indicators and ecological indicators that can be found in the literature, very often they are more or less specific for a given kind or stress or applicable to a particular type of community or scale of observation, and rarely will its wider validity have actually been proved conclusively. As will be seen through this volume, the generality of the selected indicators is only limited.

2.2 CLASSIFICATION OF ECOSYSTEM HEALTH INDICATORS

The ecological indicators applied today in different contexts, for different ecosystems, and for different problems can be classified on six levels from the most reductionistic to the most holistic indicators. Ecological indicators for EHA do not include indicators of climatic conditions, which in this context are considered entirely natural conditions.

2.2.1 Level 1

Level 1 covers the presence or absence of specific species. The best-known application of this type of indicator is the saprobien system,[6] which classifies streams into four classes according to their pollution by organic matter causing oxygen depletion:

1. Oligosaprobic water (unpolluted or almost unpolluted)
2. Beta-mesosaprobic (slightly polluted)
3. Alpha-mesosaprobic (polluted)
4. Poly-saprobic (very polluted).

This classification was originally based on observations of species that were either present or absent. The species that were applied to assess the class of pollution were divided into four groups:

1. Organisms characteristic of unpolluted water
2. Species dominating in polluted water
3. Pollution indicators
4. Indifferent species.

Records of fish in European rivers have been used to find by artificial neural network (ANN) a relationship between water quality and presence (and absence) of fish species. The result of this examination has shown that present or absent of fish species can be used as strong ecological indicators for the water quality.

2.2.2 Level 2

Level 2 uses the ratio between classes of organisms. A characteristic example is Nyggard algae index.

2.2.3 Level 3

Level 3 is based on concentrations of chemical compounds. Examples are assessment of the level of eutrophication on the basis of the total phosphorus concentration (assuming that phosphorus is the limiting factor for eutrophication). When the ecosystem is unhealthy due to too high concentrations of specific toxic substances, the concentration of one or more focal toxic compounds is, of course, a very relevant indicator. Chapter 4 gives an example where the PCB contamination of the Great North American Lakes has been followed by recording the concentrations of PCB in birds and in water. It is often important to find a concentration in a medium or in organisms where the concentration can be easily determined and has a sufficiently high value that is magnitudes higher than the detection limit, in order to facilitate a clear indication.

2.2.4 Level 4

Level 4 applies concentration of entire trophic levels as indicators; for instance, the concentration of phytoplankton (as chlorophyll-a or as biomass per m^3) is used as indicator for the eutrophication of lakes. A high fish concentration has also been applied as indicator for a good water quality or birds as indicator for a healthy forest ecosystem.

2.2.5 Level 5

Level 5 uses process rates as indicators. For instance, primary production determinations are used as indicators for eutrophication, either as maximum gC/m^2 day or gC/m^3 day or gC/m^2 year or gC/m^3 year. A high annual growth of trees in a forest is used as an indicator for a healthy forest ecosystem and a high annual growth of a selected population may be used as an indicator for a healthy environment. A high mortality in a population can, on the other hand, be used as indication of an unhealthy environment. High respiration may indicate that an aquatic ecosystem has a tendency towards oxygen depletion.

2.2.6 Level 6

Level 6 covers composite indicators, for instance, those represented by many of E.P. Odum's attributes (see Table 2.1). Examples are biomass,

Table 2.1 Differences between initial stage and mature stage are indicated; a few attributes are added to those published by Odum[7,8]

Properties	Early stages	Late or mature stage
A: Energetic		
P/R	$\gg 1$ or $\ll 1$	Close to 1
P/B	High	Low
Yield	High	Low
Specific entropy	High	Low
Entropy production per unit of time	Low	High
Exergy	Low	High
Information	Low	High
B: Structure		
Total biomass	Small	Large
Inorganic nutrients	Extrabiotic	Intrabiotic
Diversity, ecological	Low	High
Diversity, biological	Low	High
Patterns	Poorly organized	Well organized
Niche specialization	Broad	Narrow
Size of organisms	Small	Large
Life cycles	Simple	Complex
Mineral cycles	Open	Closed
Nutrient exchange rate	Rapid	Slow
Life span	Short	Long
C: Selection and homeostasis		
Internal symbiosis	Undeveloped	Developed
Stability (resistance to external perturbations)	Poor	Good
Ecological buffer capacity	Low	High
Feedback control	Poor	Good
Growth form	Rapid growth	Feedback
Growth types	R strategists	K strategists

respiration/biomass, respiration/production, production/biomass, and ratio of primary producer to consumers. E.P. Odum uses these composite indicators to assess whether an ecosystem is at an early stage of development or a mature ecosystem.

2.2.7 Level 7

Level 7 encompasses holistic indicators such as resistance, resilience, buffer capacity, biodiversity, all forms of diversity, size and connectivity of the ecological network, turnover rate of carbon, nitrogen, and energy. As will be discussed in the next section, high resistance, high resilience, high buffer capacity, high diversity, a big ecological network with a medium connectivity, and normal turnover rates, are all indications of a healthy ecosystem.

2.2.8 Level 8

Level 8 indicators are thermodynamic variables, which can be called super-holistic indicators as they try to see the forest through the trees and capture the total image of the ecosystem without the inclusion of details. Such indicators are exergy, energy, exergy destruction, entropy production, power, mass, and energy system retention time. The economic indicator cost/benefit (which includes all ecological benefits, not only the economic benefits of the society) also belong to this level.

Section 2.4 gives an overview of the application of the eight levels in chapters 3 to 15.

2.3 INDICES BASED ON INDICATOR SPECIES

When talking about indicator species, it is important to distinguish between two cases: indicator species and bioaccumulative species (the latter is more appropriate in toxicological studies).

The first case refers to those species whose appearance and dominance is associated with an environmental deterioration, as being favored for such fact, or for its tolerance of that type of pollution in comparison to other less resistant species. In a sense, the possibility of assigning a certain grade of pollution to an area in terms of the present species has been pointed out by a number of researchers including Bellan[9] and Glemarec and Hily[10], mainly in organic pollution studies.

Following the same policy some authors have focused on the presence/absence of such species to formulate biological indices, as detailed below.

Indices such as the Bellan (based on polychaetes) or the Bellan–Santini (based on amphipods) attempt to characterize environmental conditions by analyzing the dominance of species, indicating some type of pollution in relation to the species considered to indicate an optimal environmental situation.[11–12] Several authors do not advise the use of these indicators because often such indicator species may occur naturally in relatively high densities. The point is that 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.[13] Roberts et al.[16] 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. It is, however, semiquantitative as well as specific to site and pollution type. In the same way, the benthic response index[17] is based upon the type (pollution tolerance) of species in a sample, 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 example, which accounts for the presence of species indicating a type of pollution and of species indicating a nonpolluted situation, has been considered useful in terms of the application of the European Water Framework Directive to coastal ecosystems and estuaries. In fact, although this index is very much based on the paradigm of Pearson and Rosenberg[18] which emphasizes the influence of organic matter enrichment on benthic communities, it was shown to be useful for the assessment of other anthropogenic impacts, such as physical alterations in the habitat, heavy metal inputs, etc. What is more, it has been successfully applied to Atlantic (North Sea; Bay of Biscay; and south of Spain) and Mediterranean (Spain and Greece) European coasts.[14]

Regarding submarine vegetation, there is a series of genera that universally appear when pollution situations occur. Among them, there are the green algae: *Ulva*, *Enteromorpha*, *Cladophora* and *Chaetomorpha*; and the red algae: *Gracilaria*, *Porphyra* and *Corallina*.

High structural complexity species, such as Phaeophyta (belonging to Fucus and Laminaria orders), are seen worldwide as the most sensitive to any kind of pollution, with the exception of certain species of the Fucus order that can cope with moderate pollution.[19] On the other hand, marine Spermatophytae are considered indicator species of good water quality.

In the Mediterranean Sea, for instance, the presence of Phaeophyta *Cystoseira* and *Sargassum* or meadows of *Posidonia oceanica* indicate good water quality. Monitoring population density and distribution of such species allows detecting and evaluating the impact whatever activity.[20] *Posidonia oceanica* is possibly the most commonly used indicator of water quality in the Mediterranean Sea[21,22] and the conservation index,[23] based on the named marine Spermatophyta, is used in such littoral.

The description of above-mentioned indices is given below.

2.3.1 Bellan's Pollution Index[11]

$$\mathrm{IP} = \sum \frac{\text{Dominance of pollution indicator species}}{\text{Dominance of pollution/clear water indicators}}$$

Species considered as pollution indicators by Bellan are *Platenereis dumerilli, Theosthema oerstedi, Cirratulus cirratus* and *Dodecaria concharum.*

Species considered as clear-water indicators by Bellan are *Syllis gracillis, Typosyllis prolifera, Typosyllis* sp. and *Amphiglena mediterranea.*

Index values over 1 show that the community is pollution disturbed. As organic pollution increases, the value of the index goes higher, which is why (in theory) different pollution grades can be established, although the author does not fix them.

This index was designed in principle to be applied to rocky superficial substrates. Nevertheless, Ros et al. modified it in terms of the used indicator species in order to be applicable to soft bottoms.[24] In this case, the pollution indicator species are *Capitella capitata, Malococerus fuliginosus* and *Prionospio malmgremi*, and the clear water indicator species is *Chone duneri.*

2.3.2 Pollution Index Based on Ampiphoids[12]

This index follows the same formulation and interpretation as Bellan's, but is based on the amphipods group.

The pollution indicator species are *Caprella acutrifans* and *Podocerus variegatus*. The clear-water indicator species are *Hyale.* sp., *Elasmus pocllamunus* and *Caprella liparotensis.*

2.3.3 AMBI[14]

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

I. Species very sensitive to organic enrichment and present under unpolluted conditions.
II. Species indifferent to enrichment, always in low densities with nonsignificant variations with time.
III. Species tolerant to an 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 formula is as follows:

$$\mathrm{AMBI} = \frac{\begin{array}{c}(0 \times \%\mathrm{GI}) + (1.5 \times \%\mathrm{GII}) + (3 \times \%\mathrm{GIII}) \\ + (4.5 \times \%\mathrm{GIV}) + (6 \times \%\mathrm{GV})\end{array}}{100}$$

The index results are classified as:

- Normal: 0.0–1.2
- Slightly polluted: 1.2–3.2

- Moderately polluted: 3.2–5.0
- Highly polluted: 5.0–6.0
- Very highly polluted: 6.0–7.0.

For the application of this index, nearly 2000 taxa have been classified, which are representative of the most important soft-bottom communities present in European estuarine and coastal systems. The marine biotic index can be applied using the AMBI software[14] (freely available at <http://www.azti.es>).

2.3.4 Bentix[15]

This index is based on AMBI index but lies in the reduction of the ecological groups involved in the formulae in order to avoid errors in the grouping of the species and reduce effort in calculating the index:

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

Group I: This group includes species sensitive to disturbance in general.
Group II: Species tolerant to disturbance or stress whose populations may respond to enrichment or other source of pollution.
Group III: This group includes the first order opportunistic species (pronounced unbalanced situation), pioneer, colonizers, or species tolerant to hypoxia.

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

- Normal: 4.5–6.0
- Slightly polluted: 3.5–4.5
- Moderately polluted: 2.5–3.5
- Highly polluted: 2.0–2.5
- Very highly polluted: 0.

2.3.5 Macrofauna Monitoring Index[16]

The authors developed an index for biological monitoring of dredge spoil disposal. Each of the 12 indicator species is assigned a score, based primarily on the ratio of its abundance in control versus impacted samples. The index value is the average score of those indicator species present in the sample.

Index values of <2, 2–6 and >6 are indicative of severe, patchy, and no impact, respectively.

The index is site- and impact-specific but the process of developing efficient monitoring tools from an initial impact study should be widely applicable.[16]

2.3.6 Benthic Response Index[17]

The benthic response index (BRI) is the abundance weighted average pollution tolerance of species occurring in a sample, and is similar to the weighted average approach used in gradient analysis.[25,26] The index formula is:

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

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 pollutant score (p_i) for the species involves four steps:

1. Assembling a calibration infaunal data set.
2. Conducting 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.
4. Standardizing 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 calculated as:

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

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 only has been applied for assessing benthic infaunal communities on the Mayland shelf of southern California employing a 717-sample calibration data set.

2.3.7 Conservation Index[23]

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

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

Authors applied the index near chemical industrial plants. Results led them to establish four grades of *Posidonia* meadow conservation, which allow identification of increasing impact zones, as changes in the industry activity can be detected by the conservation status in a certain location.

Also, there are species classified as bioaccumulative, defined as those capable of resisting and accumulating diverse pollutant substances in their tissues, which allows their detection when they are present in the environment at such low levels (and are therefore difficult to detect using analytical techniques).[27]

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.

Molluscs, specifically the bivalve class, have been one of the most commonly used species in determining the existence and quantity of a toxic substance.

Individuals of the genres *Mytilus*,[28–37] *Cerastoderma*,[38–40] *Ostrea*[35,41] and *Donax*[42,43] are considered to be ideal for research involving the detection of the concentration of a toxic substance in the environment, due to their sessile nature, wide geographical distribution, and capability to detoxify when pollution ceases. In that sense, Goldberg et al.[29] introduced the concept of "mussel watch" when referring to the use of molluscs in the detection of polluting substances, due to their wide geographical distribution and their capability of accumulating those substances in their tissues. The National Oceanic and Atmospheric Agency (NOAA) in the U.S. has developed a "mussel watch" program since 1980 focusing on pollution control along the North American coasts. There are programs similar to the North American one in Canada,[31,44] the Mediterranean Sea,[45] the North Sea[46] and on the Australian coast.[47–49]

Likewise, certain species of the amphipods group are considered capable of accumulating toxic substances,[50,51] as well as species of the polychaetes group like *Nereis diversicolor*,[52,53] *Neanthes arenaceodentata*,[54] *Glycera alba*, *Tharix marioni*,[55] or *Nephtys hombergi*.[56]

Some fish species have also been used in various work focusing on the effects of toxic pollution of the marine environment, due to their bioaccumulative capability[57–59] and the existing relationship among pathologies suffered by any benthic fishes and the presence of polluting substances.[60–62]

Other authors such as Levine,[63] Maeda and Sakaguchi,[64] Newmann et al.,[65] and Storelli and Marcotrigiano[66] have looked into algae as indicators for the presence of heavy metals, pesticides and radionuclides. *Fucus*, *Ascophyllum* and *Enteromorpha* are the most utilized.

For reasons of comparison, the concentrations of substances in organisms must be translated into uniform and comparable units. This is done through the ecologic reference index (ERI), which represents a potential for environmental effects. This index has only been applied using blue mussels:

$$\mathrm{ERI} = \frac{\text{Measured concentration}}{\mathrm{BCR}}$$

Table 2.2 Upper limit of BCR for hazardous substances in blue mussel according to OSPAR/MON (1998)

Substance	Upper limit of BCR value (ng/g dry weight)
Cadmium	550
Mercury	50
Lead	959
Zinc	150,000

where BCR is the value of the background/reference concentration (see Table 2.2).

Few indices (such as the latter) based on the use of bioaccumulative species have been formulated, most of which involve the simple measurement of the effects (e.g., percentage incidence or percentage mortality) of a certain pollutant on those species, or the use of biomarkers (which can be useful to scientists evaluating the specificity of the responses to natural or anthropogenic changes). However, it is very difficult for the environmental manager to interpret increasing or decreasing changes in biomarker data.

The Working Group on Biological Effects of Contaminants (WGBEC) in 2002 recommended different techniques for biological monitoring programs (see Table 2.3).

2.4 INDICES BASED ON ECOLOGICAL STRATEGIES

Some indices try to assess environmental stress effects accounting for the ecological strategies followed by different organisms. That is the case of trophic indices such as the infaunal index proposed by Word,[67] which are based on the different feeding strategies of the organisms. Another example is the nematodes/copepods index[68] which account for the different behavior of two taxonomic groups under environmental stress situations. However, several authors have rejected them due to their dependence on parameters such as depth and sediment particle size, as well as because of their unpredictable pattern of variation depending on the type of pollution.[69,70] More recently, other proposals have appeared, such as the polychaetes/amphipods ratio index, or the index of r/K strategies, which considers all benthic taxa although the difficulty of scoring exactly each species through the biological trait analysis has been emphasized.

Feldman's R/P index, based on marine vegetation, is often used in the Mediterranean Sea. It was established as a biogeographical index and it is based on the fact that *Rodophyceae* sp. number decreases from the tropics to the poles. Its application as an indicator holds on the higher or lower sensitivity of *Phaeophyceae* and *Rhodophyceae* to disturbance.

Table 2.3 Review of different techniques for biological monitoring

Method	Organism	Issues addressed	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 Bivalve molluscs	Organophosphates and carbonates or similar molecules	Measures exposures	−2.5 × reference site
Metallothionein induction	Fish *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 metabolise 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
Lysososmal stability	Fish *Mytilus* sp.	Not contaminant specific but responds to a wide variety of xenobiotics contaminants and 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 contaminants and metals	Provides a link between exposure and pathological endpoints	2.5 × reference site

(*Continued*)

Table 2.3 Continued

Method	Organism	Issues addressed	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 in coastal waters	2.0 × reference site or 20% change
Reproductive success in fish	Zoarces viviparous	Not contaminant-specific, will respond to a wide of environmental contaminants	Measures reproductive output and survival of eggs and fry in relation to contaminants	

2.4.1 Nematodes/Copepods Index[68]

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

$$\mathrm{I} = \frac{\text{Nematode abundance}}{\text{Copepod abundance}}$$

Values of such a ratio can increase or decrease according to levels of organic pollution. This happens by means of a different response of those groups to the input of organic matter to the system. Values of over 100 demonstrate a high organic pollution.

According to the authors, the index application should be limited to certain intertidal zones. In infralittoral areas at certain depths, despite the absence of pollution, the values obtained were very high. The explanation for this is the absence of copepods at such depths, possibly due to a change in the optimal interstitial habitat for that taxonomic group (see Reference 68).

2.4.2 Polychaetes/Amphipods Index

This index is similar to the nematodes/copepods, but is applied to the macrofauna level using the polychaetes and amphipods groups. The index was formerly designed to measure the effects of crude oil pollution:

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

The index values are classified as: $I \leq 1$ = nonpolluted and $I > 1$ = polluted.

2.4.3 Infaunal Index[67]

The macrozoobenthos species can be divided into:

1. Suspension feeders
2. Interface feeders
3. Surface deposit feeders
4. Subsurface deposit feeders.

Based on this division, the trophic structure of macrozoobenthos can be determined using the following equation:

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

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. Near a value of 0 subsurface, feeders are dominant, meaning that the environment is probably heavily disturbed due to human activities.

One of the disadvantages of a trophic index is the determination of the diet of the organisms, which can be developed through the study of the stomach content or in laboratory experiments. Generally, the real diet (i.e., the one studied observing the stomach content) is difficult to establish, and can vary from one population to another among the same taxonomic entity. For example, *Nereis virens* is an omnivore species along the European coast but a herbivore along the North American coast.[71]

Another aspect to be considered when determining the trophic category of many polychaetes species, is their alternative feeding behavior that can appear under certain circumstances. Buhr (1976) determined, through laboratory experiments, that the terebellid *Lanice conchylega*, considered as a detritivore, changes into a filterer when a certain concentration of phytoplankton is present in the water column Taghon et al. (1980)[72] observed that some species of the Spionidae family, usually taken for a detritivore, could change into a filterer, modifying the mandibular palps into a characteristic helicoidal shape.

On the other hand, some species of the Sabellidae and Owenidae families can change from filterers to detritivores. Some limnivores and detritivores can be considered carnivores when they consume the remains of other animals.[73]

Those facts nowadays lead to doubts about the existence of a clear separation among such diverse feeding strategies. This is why other characteristics such as the grade of individual's mobility and the morphology of the mouth apparatus intervene in the definition of the trophic category of polychaetes.[74] The different combinations of that set of characteristics are what Fauchald and Jaumars term "feeding guilds."[71]

Authors such as Maurer et al.[75] and Pires and Múniz[76] have tried the use of the classification of the different polychaetes species in feeding guilds when studying the structure of the benthic system and when identifying the different impacts, both with good results.

The main problem when using such a classification is without doubt the difficulty that carries the determination of each one of those combinations for each species. According to a study by Dauer, 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 monospecific every combination (Dauer et al., 1981). This leads us to believe that such a classification very often does not make much sense from a practical point of view.

2.4.4 Feldman Index

$$\mathrm{I} = \frac{N^{\circ}\ \text{Species of } \textit{Rhodophyceae}}{N^{\circ}\ \text{Species of } \textit{Phaeophyceae}}$$

Cormaci and Furnari detected values of over 8 in polluted areas in southern Italy,[77] when normal values in a balanced community oscillate between 2.5 and 4.5. Verlaque studied the effects of a thermal power station,[78] and also found higher values of those the index, but considers due to the presence of communities with higher optimum temperature.

However, Belsher and Bousdouresque analyzed vegetation in small harbors and found that as the Phaeophyceae increases, the index decreases.[79]

2.5 INDICES BASED ON THE DIVERSITY VALUE

Diversity is the other mostly used concept, focusing on the fact that the relationship between diversity and disturbances can be seen as a decrease in the diversity when the disturbances increase.

Magurran divides the diversity measurements into three main categories:[80]

1. Indices that measure the enrichment of the species, such as Margalef, which are, in essence, a measurement of the number of species in a defined sampling unit.
2. Models of the abundance of species, as the *K*-dominance curves[70] or the log-normal model,[81] which describe the distribution of their abundance, going from those that represent situations in which there is a high uniformity to those that characterize cases in which the abundance of the species is very unequal. However, the log-normal model deviation was rejected once ago by several authors due to the impossibility of finding any benthic marine sample that clearly responded to the log-normal distribution model.[70,82,83]
3. Indices based on the proportional abundance of species that pretend to solve enrichment and uniformity in a simple expression. Such indices can also be divided into those based on statistics, information theory, and dominance indices. Indices derived from 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 are referred to as measurements that mostly ponder the abundance of common species instead of the enrichment of the species.

Meanwhile, average taxonomic diversity and distinctness measures has been used in some research to evaluate biodiversity in the marine environment,[84–86] as it takes into account taxonomic, numerical, ecological, genetic, and filogenetic aspects of diversity. These measures address some of the problems identified with species richness and the other diversity indices.[85]

2.5.1 Shannon–Wiener Index[87]

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.

The index takes the form:

$$\mathrm{H}' = -\sum p_i \log 2p_i$$

where p_i is the proportion of individuals found in the 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 is the number of individuals of the species i and N is the 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 take values between 0 and 5. Maximum values are rarely over 5 bits per individual. Diversity is a logarithmic measurement which makes it, to a certain extent, a sensitive index in the range of values next to the upper limit.[88]

As an ordinary basis, in the literature, low index values are considered to be indication of pollution.[89–98]

However, one of the problems arising with its use is the lack of objectivity when establishing as a precise manner from what value it should start detecting the effects of such pollution.

Molvaer et al.,[99] established the following relationship between the indices and the different ecological levels according to what is recommended by the Water Framework Directive:

- High status: >4 bits/individual
- Good status: 4–3 bits/individual
- Moderate status: 3–2 bits/individual
- Poor status: 2–1 bits/individual
- Bad status: 1–0 bits/individual.

Detractors of Shannon index base their criticisms on its lack of sensitivity when it comes to detecting the initial stages of pollution.[18, 100–101]

Gray and Mirza,[102] in a study on 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 and Cardell,[103] in their study on 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 explanation to that statement, set out a new structural index proposal, the lack of applicability of which has already been demonstrated by Salas.[104]

2.5.2 Pielou Evenness Index

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

where $\mathrm{H}'_{\max}$ is the maximum possible value of Shannon diversity and S is the number of species.

The index oscillates from 0 to 1.

2.5.3 Margalef Index

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

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

where S is the number of species and N is the total number of individuals. The author did not establish reference values.

The main problem that arises when applying this index is the absence of a limit value, therefore it is difficult to establish reference values. Ros and Cardell[103] consider values below 4 as typical of polluted. Bellan-Santini,[12] on the contrary, established that limit when the index takes values below 2.05.

2.5.4 Berger–Parker Index

The index expresses the proportional importance of the most abundant species, and takes this shape:

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

where $n_{\max}$ is the number of individuals of the one most abundant species and N is the total number of individuals. The index oscillates from 0 to 1 and, in contrast with the other diversity indices, high values show a low diversity.

2.5.5 Simpson Index

Simpson defined their index on the probability that two individuals randomly extracted from an infinitely large community could belong to the same species:[105]

$$\mathrm{D} = \sum p_i^2$$

where p_i is the individuals proportion of the species i. To calculate the index for a finite community use:

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

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

Like the Berger–Parker index, this one oscillates from 0 to 1, it has no dimensions and similarly, the high values imply a low diversity.

2.5.6 Deviation from the Log-Normal Distribution[102]

This method, proposed by Gray and Mirza in 1979, is based on the assumption that when a sample is taken from a community, the distribution of the individuals tends to follow a log-normal model.

The adjustment to a logarithmical normal distribution assumes that the population is ruled by a certain number of factors and it constitutes a community in a steady equilibrium; meanwhile, the deviation from such distribution implies that any perturbation is affecting it.

2.5.7 *K*-Dominance Curves[106]

The *K*-dominance curve is the representation of the accumulated percentage of abundance vs. the logarithm of the sequence of species ordered 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 too.

2.5.8 Average Taxonomic Diversity[84]

This measure, equal to taxonomic distinctness, is based on the 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 and the second from species *j*).

It is 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:

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

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

2.5.9 Average Taxonomic Distinctness[84]

To remove the dominating effect of the species abundance distribution, Warwick and Clarke[84] proposed to divide the average taxonomic diversity index by the Simpson index, giving the average taxonomic distinctness index:

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

when quantitative data is not available and the sample consists simply of a species list (presence/absence data) the average taxonomic distinctness takes the following form:

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

where *S*, as usual, is the observed number of species in the sample and the double summation ranges over all pairs *i* and *j* of the species ($i < j$).

Taxonomic distinctness is reduced in respect to increasing environmental stress and this response of the community lies at the base of this index concept. Nevertheless, it is most often very complicated to meet certain requirements to apply it, as having a complete list of the species present in the area under study in pristine situations. Moreover, some works, have shown that in fact taxonomic distinctness is not more sensitive than other diversity indices usually applied when detecting disturbances,[107] and consequently this measure has not been widely used on marine environment quality assessment and management studies.

2.6 INDICATORS BASED ON SPECIES BIOMASS AND ABUNDANCE

Other approaches account for the variation of organism's biomass as a measure of environmental disturbances. Along these lines, there are methods such as SAB,[18] 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 that impedes relating it quantitatively to the various environmental factors. The ABC method[108] also involves the comparison between the cumulative curves of species biomass and abundance, from which Warwick and Clarke[85] derived the *W*-statistic index.

2.6.1 ABC Method[108]

This method is based on the idea that the distribution of a number of individuals for the different species in the macrobenthos communities is different to the biomass distribution.

It is adapted from the *K*-dominance curve already mentioned, showing in one graphic the *K*-dominance and biomass curves. The graphics are made up comparing the interval of species (in the abscise axis), decreasingly arranged and in logarithmical scale, to the accumulated dominance (in the ordinate axis).

According to the range of disturbance, three different situations can be given:

1. In a system with no disturbances, a relatively low number of individuals contribute to the major part of the biomass, and at the same time, the distribution of the individuals among the different species is similar. The representations would show the biomass curve above the dominance one, indicating higher numeric diversity than biomass.
2. Under moderate disturbances, there is a decrease in the dominance as regards biomass; however, abundances increase. The graphic shows both curves intersected.
3. In the case of intense disturbances, the situation is totally the opposite, and only a few species monopolize the greater part of the individuals,

> which are of a small size, which is why the biomass is low and is more equally shared. It can be seen in the representation how the curve of the number of individuals is placed above the biomass curve, indicating a higher diversity in the biomass distribution.

Some studies have tried to lead this method into a measurable index,[109–112] with the study by Clarke being the most commonly accepted one:[110]

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

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 nondisturbed system (high status) to −1, which defines a polluted situation (low status). Values close to 0 indicate a moderate level of pollution (moderate status).

The method is specific of organic pollution and it has been applied, with satisfactory results, to soft-bottom tropical communities,[113,114] to experiments,[115] to fish-factoring disturbed areas,[116] and on coastal lagoons.[117,118] However, several studies obtained confusing results after applying that technique to estuarine zones,[109,119–122] induced by the appearance of dominant species in normal conditions and favored by different environmental factors.

Although it is a method designed to be applied to benthic macrofauna, Abou-Aisha et al.[123] used it to detect the impact of phosphorus waste in macroalgae, in three areas of the Red Sea. In spite of that, the problem when applying it to marine vegetation lies on the difficulty of counting the number of individuals in the vegetal species.

2.7 INDICATORS INTEGRATING ALL ENVIRONMENT INFORMATION

From a more holistic point of view, some studies proposed indices capable of at least trying to integrate the whole environmental information. A first approach for application in coastal areas was developed by Satmasjadis,[124] relating sediment particles size to benthic organisms diversity. Wollenweider et al.[125] developed a trophic index (TRIX) integrating chlorophyll-a, oxygen saturation, total nitrogen, and phosphorus to characterize the trophic state of coastal waters.

In a progressively more complex way, other indices such as the index of biotic integrity (IBI) for coastal systems,[126] the benthic index of environmental condition,[96] or the Chesapeake Bay B-BI index[127] included physicochemical factors, diversity measures, specific richness, taxonomical composition, and the trophic structure of the system.

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) combines two separate measures (health and importance) into a single index. The estuarine fish health index (FHI) is based on both qualitative and quantitative comparisons with a reference fish community.[128] The estuarine biotic integrity index (EBI)[129] 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 generalized way because they have usually been developed for application in a particular system or area, which makes them dependent on seasonality and the type of habitat. On the other hand, they are difficult to apply as they need a large amount of data of different nature.

2.7.1 Trophic Index[125]

$$\mathrm{TRIX} = \frac{k}{n} \times \sum (M_i - L_i)/(U_i - L_i)$$

in which $k = 10$ (scaling the result between 0 and 10), $n = 4$ (number of variables are 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 limit chosen and indicate how close the current state is to the natural state. However, comparing TRIX values of different areas becomes more difficult. When a wider, more general range is used for the limits, TRIX values for different areas can more easily be compared to each other.

2.7.2 Coefficient of Pollution[124]

Calculation of the 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 is the coefficient of pollution, S' is the sand equivalent, s is the percent sand, t is the percent silt, i_0 is the theoretical number of individuals, i is the number of individuals, h is the station depth, g' is the theoretical number of species, and g is the number of species.

2.7.3 Benthic Index of Environmental Condition[96]

Benthic index $= (2.3841 \times$ Proportion of expected diversity$) + (-1.6728 \times$ Proportion of total abundance as tubifids$)$ $+$ $(0.6683 \times$ Proportion of total abundance as bivalves$)$.

The expected diversity is calculated throughout Shannon–Wiener index adjusted for salinity:

$$\text{Expected diversity} = 0.75411 + (0.00078 \times \text{salinity}) + (0.00157 \times \text{salinity}^2) + (-0.00078 \times \text{salinity}^3)$$

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 nondegraded or reference conditions.

The final development of the index involved calculating discriminating scores for all samples sites and normalizing calculated scores to a scale of 0 to 10, setting the break point between degraded and nondegraded reference sites at 4.1. So the index values lower than 4.1 indicate degraded conditions, higher values than 6.1 indicate nondegraded situations, and values between 6.1 and 4.1 reveal moderate disturbance.

2.7.4 B-IBI[127]

Eleven metrics are used to calculate the B-IBI[127]

1. Shannon–Wiener species diversity index
2. Total species abundance
3. Total species biomass
4. Percent abundance of pollution-indicative taxa
5. Percent abundance of pollution-sensitive taxa
6. Percent biomass of pollution-indicative taxa
7. Percent biomass of pollution-sensitive taxa
8. Percent abundance of carnivore and omnivores
9. Percent abundance of deep-deposit feeders
10. Tolerance Score
11. Tanypodinae to Chironomidae percent abundance ratio.

The scoring of metrics to calculate the B-IBI is done by comparing the value of a metric from the sample of unknown sediment quality to thresholds established from reference data distributions.

This index was developed to establish ecological status of Chesapeake Bay and it is specific to habitat type and seasonality, its use advisable only during spring.

2.7.5 Biotic Integrity (IBI) for Fishes

A fish index of biotic integrity (IBI) was developed for tidal fish communities of several small tributaries to the Chesapeake Bay.[130,131]

Nine metrics are used to calculate the index having in account species richness, trophic structure and abundance:

1. Number of species
2. Number of species comprising 90% of the catch
3. Number of species in the bottom trawl
4. Proportion of carnivores
5. Proportion of planktivores
6. Proportion of benthivores
7. Number of estuarine fish
8. Number of anadromous fish
9. Total fish with Atlantic menhaden removed.

The scoring of metrics to calculate the index is done by comparing the value of a metric from the sample of unknown water quality to thresholds established from reference data distributions.

2.7.6 Fish Health Index (FHI)[128]

This index is based on the community degradation index (CDI), which measures the degree of dissimilarity (degradation) between a potential fish assemblage and the actual measured fish assemblage.

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

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

where J is the number of species in the system divided by the number of species in the reference community, $P =$ is the potential species richness (number of species) of each reference community, and $P_{\max}$ is 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.[128] Although the index has proved to be a useful tool in condensing information of estuarine fish assemblages into a single numerical value, the index is only based on presence/absence data and does not take into account the relative proportions of the various species present.

2.7.7 Estuarine Ecological Index (EBI)[129]

The EBI includes the following eight metrics:

1. Total number of species
2. Dominance
3. Fish abundance
4. Number of nursery
5. Number of estuarine spawning species
6. Number of resident species
7. Proportion of benthic associated species
8. Proportion of abnormal or diseased fishes.

The usefulness of this index requires it to reflect not only the current status of fish communities but also to be applicable over a wide range of estuaries, although this is not entirely achieved.[132]

2.7.8 Estuarine Fish Importance Rating (FIR)[133]

This index is constructed from seven weighted measures of species and estuarine importance and is designed to work on a presence/absence data set where species are only considered to be present if they constituted more than 1% of any catch by number.

Measures of species importance:

- Number of exploitable species
- Number of estuarine-dependent species
- Number of endemic species.

Measures of estuarine importance:

- Type
- Size
- Condition
- Isolation.

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.

2.8 PRESENTATION AND DEFINITION OF LEVEL 7 AND 8 INDICATORS — HOLISTIC INDICATORS

An ecological network is often drawn as a conceptual diagram that is used as the first step in a modeling development procedure. Figure 2.1 shows a nitrogen cycle in a lake and it represents a conceptual diagram and the ecological network for a model of the nitrogen cycle. The complexity of the ecological network in Figure 2.1 cannot be used as ecological indicator because the real network is simplified too much in the figure; but if observations of the real network make it possible to draw close to the real network, a similar figure is obtained; but much more complicated. The complexity of the network in this figure could be used as an indicator for the function of the real ecosystem — even if the network was still a simplification of the real ecosystem.

Gardner and Ashby examined the influence on stability of connectivity (defined as the number of food links in the food web as a fraction of the number of topologically possible links) of large dynamic systems.[134] They suggest that all large complex dynamic systems may show the property of being stable up to a critical level of connectivity and then as the connectivity increases further, the system suddenly goes unstable. A connectivity of about 0.3 to 0.5 seems to give the highest stability.