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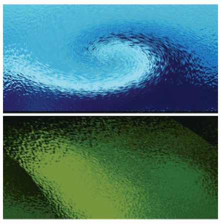

REVIEW

Biotic indices for ecological status of transitional water ecosystems

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Abstract

1 - The biological elements, proposed by the WFD as quality elements for the classification of ecological status of the transitional waters are: composition, abundance and biomass of phytoplankton; composition and abundance of other aquatic flora (i.e. macroalgae and angiosperms); composition and abundance of benthic invertebrate fauna; composition and abundance of fish fauna.

2 - Although the directive proposed these biological elements, it doesn't provide clear indication on the "biocriteria" to achieve. WFD suggested that ecosystem health should be defined by comparison to reference conditions.

3 - Recently, there has been a growing interest and need for sound and robust ecological indices to evaluate transitional ecosystem status and condition, mainly under the scope of the Water Framework Directive implementation.

4 - A good biotic index should reflect the biological integrity; respond to environmental stresses in monotonic way; be measurable with low error; be cost-effective; be not invasive (i.e. the measure methods do not significantly disturb or alter habitats and biota).

5 - Several indices have been developed for environmental quality assessment using macrobenthic assemblages, ranging from the comparison of a single metric to complex multivariate analyses. Some indices were developed for fish assemblages and for macrophytes, but only few indices were proposed considering the phytoplankton.

6 - In this report, we shown and compare several biotic indices used for evaluating ecological status of transitional water ecosystems using the five biological quality elements.

Keywords: Ecological indicators, WFD, Biological quality elements, Biocriteria, Transitional waters

Contents

1 Introduction

Transitional waters, which include coastal lagoons, salt marshes, saltworks, river estuaries and deltas, are heterogeneous and dynamics ecosystems (Gomez *et al.*, 1998; Benedetti-Cecchi *et al.*, 2001). Their morphology and hydrology change quickly under the influence of high sedimentation rate, natural coastal dynamics and frequent human intervention (Ver *et al.*, 1999; Pastres *et al.*, 2004). These aquatic habitats are characterised by high trophic flux and large variation of chemical and physical characteristics and fast biogeochemical cycles (Herbert, 1999; Petihakis *et al.*, 1999; De Wit *et al.*, 2001). Moreover most transitional water ecosystems are very vulnerable to eutrophication and microbial and chemical pollution because of their confinement, shallow depth and reduced water exchange (Barnes, 1999). All of these lead to rapid and often unpredictable changes in communities' composition and functioning (Herbert, 1999; Sfriso *et al.*, 2001; Mistri *et al.*, 2002).

Conservation and management of transitional waters require monitoring activities that integrate chemical and physical evaluations with biological assessment (Gibson *et al.*, 2000; Logan and Furse, 2002). This approach was recently introduced in some national and international legislations, which include the US Clean Water Act (33 U.S.C. ss/1251 et seq., 1977) and the European Water Framework Directive (WFD; European Commission, 2000). The biological elements, proposed in the WFD as quality elements for the classification of ecological status of the transitional waters, are:

- composition, abundance and biomass of

phytoplankton;

- composition and abundance of other aquatic flora (i.e. macroalgae and angiosperms);
- composition and abundance of benthic invertebrate fauna;
- composition and abundance of fish fauna.

Although the directive proposed these biological elements, it doesn't provide clear indication on the "biocriteria" to achieve. The biocriteria are guidelines or benchmarks to be adopted to evaluate the relative biological integrity of surface waters; they could be defined as "narrative expressions or numerical values that describe the biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use" (USEPA, 1990). WFD suggested that ecosystem health should be defined by comparison to reference conditions. Reference sites should be represented by "undisturbed habitats", which have "biological integrity". The biological integrity could be defined as "...the condition of the aquatic community inhabiting unimpaired water bodies of a specified habitat as measured by community structure and function" (USEPA, 1990).

Since absolutely pristine transitional waters probably do not exist (Basset and Abbiati, 2004; Thompson and Lowe, 2004), managers must decide an acceptable levels of minimum impacts that exist or that are achievable in their region, taking in to account environmental conditions like salinity gradients, trophic state, bottom sediment types, morphology and biological communities (Gibson *et al.*, 2000). In practice, minimally impaired sites, which are not necessarily pristine, could represent reference condition; references sites should, however, exhibit minimal influence by human activities relative to the overall region of study (Reynoldson *et al.*, 1997). Unlikely even minimally impaired transitional water ecosystems do not exist within most of the regions, especially along the European coasts. In these cases, some authors (Gibson *et al.*, 2000) suggest to adopt different approaches, like:

- comparisons with historical data that provide information about the communities that once existed and/or those that may be re-established;
- application of statistical or empirical models following from first principles and assumptions and/or built from observed relationships between variables;
- turn to opinion/consensus of qualified experts.

Alternatively, from an anthropogenic point of view, the biocriteria could be established on the basis of the use designation of the ecosystem (e.g. recreational activities, fishing, aquaculture, harbour, etc.; Fig. 1).

The assessment of ecosystem health required both a good knowledge of the ecosystem properties and functioning, and some adequate tools to measure the alteration from the reference or desirable condition. Appropriate biotic indicators and indices could represent these tools. In this context biotic indices, based on the biological components of the ecosystems, were specifically developed with the aims to provide integrate and effectiveness information on the ecosystem health. A number of ecological indicators have been applied to the "Ecosystem Health Assessment" (EHA) but broad indicators that provide unambiguous information towards different anthropogenic disturbs and in different habitats do not exist yet (Simon, 2000; Jørgensen *et al.*, 2005b).

Biotic indices are based on different approaches including species diversity, species sensitivities to disturbance, reproductive and trophic strategies, etc. They measure biotic attributes and should provide quantitative information on ecological condition, structure and functioning of ecosystems. To be useful for environmental quality assessment and management the indices required the definition of relative or absolute interpretative scales.

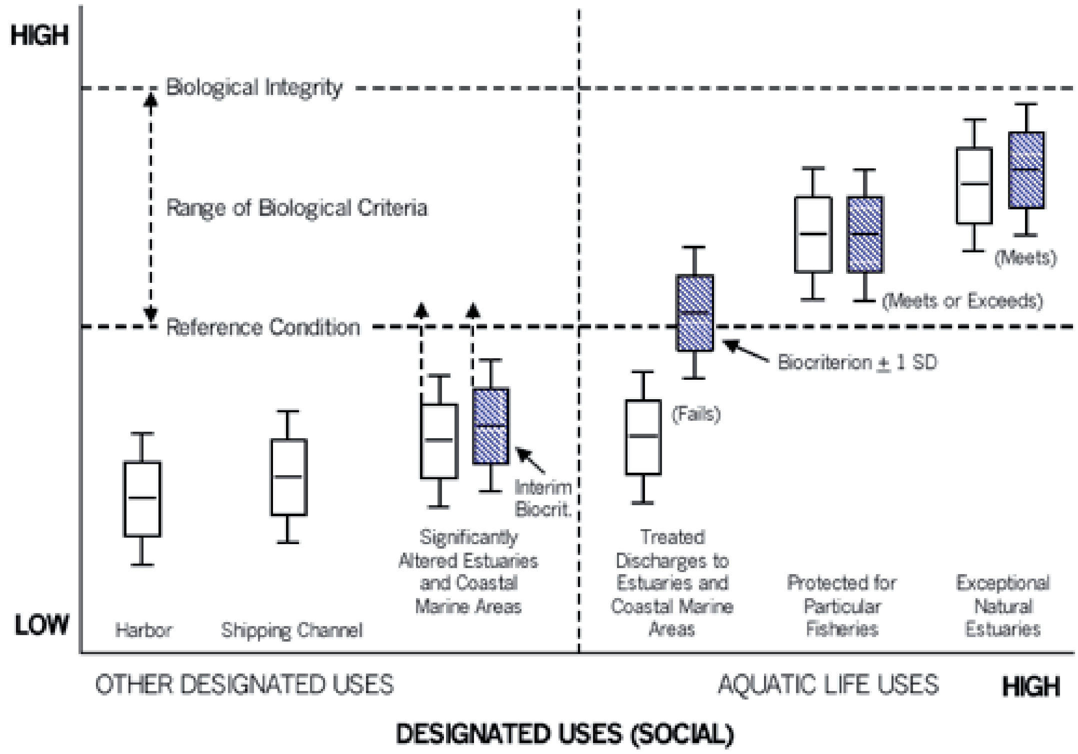

Figure 1. Example of general biocriteria for given classifications of estuaries and coastal marine areas (from Gibson et al., 2000).

1.1 Characteristics of a good biological quality element

Biological quality elements to be use as environmental indicators and adopted in biotic indices should be (Ward and Jacoby, 1992):

- easily and quickly measurable;
- wide distributed and present in both impaired and pristine areas;
- response in a predictable way to natural and anthropogenic disturb;
- reflect the quality of the other components of the environment.

1.2 Characteristics of a good biotic index

A good biotic index should (Gibson *et al.*, 2000):

- reflect the biological integrity;
- respond to environmental stresses in monotonic way;
- be measurable with low error;
- be cost-effective;
- be not invasive (i.e. the measure methods do not significantly disturb or alter habitats and biota).

1.3 Biological quality elements

Several indices have been developed for environmental quality assessment using macrobenthic assemblages, ranging from the comparison of a single metric to complex multivariate analyses. Some indices were developed for fish assemblages and, recently, for macrophytes, but only few indices were proposed considering the phytoplankton, especially in transi¬tional waters.

1.3.1 Benthic macroinvertebrates

The benthic macroinvertebrates(macro-invertebrates are generally defined as organism retained by a 0.5 mm sieve size) inhabiting soft bottoms are more sedentary and thus more reliable as site indicators of water quality over time compared to fish and plankton. Benthic assemblages are potentially good indicators for the following characteristics (Bilyard, 1987; Dauer, 1993):

- they includes several infaunal species that are typically sedentary (reduced mobility), they cannot avoid deteriorating water and sediments quality conditions and therefore are most likely to respond to local environmental impacts;
- since most species have relatively long life spans then they over time integrate disturbance events;
- they includes a broad range of taxonomic, functional and trophic groups with different tolerances to different source of disturbance;
- most species are sensitive to disturbances of habitat such that the communities respond fairly quickly with changes in species composition and abundance;
- benthic fauna are important components of the food chain, including both detritus and grazing, and often act to transport not only nutrients, but also toxicants, to the rest of the system;
- some species are commercially important or are important food source for economically important species.

From a practical point of view, benthic communities present some advantages:

- provides an "*in situ*" measure of relative biotic integrity and habitat quality;
- some species are wide distributed allowing comparison on geographical scale;
- assemblages are easily to sample and preserve.

Some limitations of benthic fauna sampling include:

- often require high taxonomic expertise;
- generally show high small spatial scale heterogeneity, then require several replicate samples;
- life cycle induce important seasonal and annual changes in the species composition and individual size;
- the cost and effort to sort, count, and identify benthic invertebrate samples can be significant, requiring tradeoffs between expenses and the desired level of confidence in decisions based upon the collected data.

1.3.2 Aquatic macrophytes (macroalgae and angiosperms)

Benthic macrophytes comprise evolutionary primitive plants like *Ulva*, *Enteromorpha*, *Gracilaria* (macroalgae) and evolutionary advanced flowering plants like *Ruppia*, *Zostera* (angiosperms or seagrasses).

Excess of nutrients in water coupled with changes in coastal hydrography and water optical properties or interactions between them often stimulate growth of macroalgae and phytoplankton which in turn increase water turbidity and shading. The result is a non-linear and self-accelerating chain reaction (Duarte, 1995; Schramm, 1999; Cloern, 2001), which shifts the dominance of primary producers in cost of seagrasses often ending in anoxia and fish killings (Schramm and Nienhuis, 1996).

There are many advantages of using benthic macrophytes as biomonitors of transitional waters:

- macrophytes are key structural and functional components of most ecosystems;
- photosynthetic sessile organisms behaving as ecosystem engineers by providing substrate, habitat and shelter for animals;
- canopy of leaves diminishes wave energy and currents significantly affecting sediment stability and retention of particles;
- macrophytes are vulnerable and adaptive to environmental stress of water and

sediment (especially for seagrasses);
- they response to pollution as well as to other kinds of disturbance, e.g. dredging, trawling, increased turbidity;
- field collections need simple equipments;
- remote means such as aerial photography, if the water is clear or shallow, can easily assess macrophyte abundance and extent;
- predictive models and sound ecological theory explain interactions to certain environmental factors, e.g. nutrients.

Expertise requirement for taxonomic identification at the species level and ephemeral behaviour with high spatial and temporal variability may disadvantage the usage of macroalgae as quality elements. However, low macroalgal diversity in transitional waters as well as easily recognizable macroalgal functional characteristics help to develop user friendly and cost-effective monitoring protocols.

Slow changes in community structure and biomass, occasionally caused by extreme meteorological (storms) and hydrological (river floods) events, may disadvantage the usage of angiosperms as a quality elements. Where long-term data series are available, one can often identify regular patterns, which are site or region-specific (e.g., biomass peaks, flowering periods, etc.). Since submerged macrophytes could also follow long-term periodicity additional parameters (e.g. water and sediment nutrient concentrations, light attenuation) are required to interpret macrophyte data. In the past, seagrasses diseases, e.g. in *Zostera*, have been also observed.

1.3.3 Phytoplankton

Phytoplankton is an important component in transitional waters both in term of biomass and primary production, which implies that this assemblage should provide valuable information in an assessment of ecosystem condition. Advantages of using phytoplankton include:

- it provide the most notable indication of eutrophication in term of rapid changes in community structure and functioning;
- changes in plankton primary production will in turn affect higher trophic levels of macroinvertebrates and fish;
- many countries routinely monitor chlorophyll as a part of water quality monitoring due to the ease and relatively low cost of analysis;
- plankton have generally short life cycles and rapid reproduction rates making them valuable indicators of short-term impacts.

The possible disadvantages using phytoplankton as bio-indicator are:

- populations are subject to rapid drift with the winds, tides, and currents, especially in the channels;
- taxonomic identification can be difficult and time-consuming;
- increased phytoplankton biomass could be balanced by increasing of grazing by zooplankton, that suggest to investigate phytoplankton and zooplankton together;
- phytoplankton exhibit high turnover rates and moreover microalgal blooms could be ephemeral, therefore high frequency sampling is required.

1.3.4 Fish

Fish are an important component of transitional ecosystems because of their economic, recreational and ecological roles. Many anthropogenic disturbs can have a direct influence on the food resources, distribution, diversity, breeding, abundance, growth, survival and behaviour of both resident and migrant fish species (Whitfield and Elliott, 2002). Despite the fact that ichthyofaunal composition in estuaries is usually dynamic, reflecting the ever changing environmental factors and life history patterns of the various species, fish communities or some selecting fishes as bio-indicators could be included in transitional water monitoring and management programmes (Whitfield and Elliott, 2002). Fish could represent good indicators of ecological health because (Attrill and Depledge, 1997; Gibson *et al.*, 2000):

- they are relatively sensitive to most habitat disturbances and may exhibit physiological, morphological, or behavioural responses to stresses;
- fish may exhibit obvious external anatomical pathology due to chemical pollutants;
- fish are important in the linkage between benthic and pelagic food webs;
- they are long-lived and are therefore good indicators of long-term effects.

Unlikely fish assemblages have some disadvantages:

- being mobile, sensitive fish species may avoid stressful environments, reducing their exposure to toxic or other harmful conditions;
- fish represent a relatively high trophic level, therefore could be not an earlier indication of water quality problems;
- monitoring fish assemblages must be take into account their life cycle and behaviour, including reproductive and overwintering migrations;
- fish studies may be biased because of recreational and commercial fishing pressures on the same or related fish assemblages;
- some fish are very habitat selective and their habitats may not be easily sampled;
- since they are mobile, spatial variability is very high, requiring a large sampling effort to adequately characterize the fish assemblage.

1.3.5 *Other possible biological elements*

The other biological elements that could be considered in the biological quality assessment are zooplankton, epibenthos living on hard bottoms and infaunal meiobenthos (practically defined as organism which size is in the range 0.063 - 0.500 mm) . As previously mentioned, zooplankton should be study coupled with phytoplankton and, possibly, with his mainly predators that is the fish. Since hard bottoms are limited distributed in transitional waters, epibenthos can not be useful everywhere, although some authors suggest to include in the monitoring programs species living on artificial hard substrata like wood piles, bridge pillar, and so on (Jan *et al.*, 1994; Marchini *et al.*, 2004). On the hard bottoms could be sample both sessile and vagile species, including several typically soft-bottom species, which here found shelter and sediment niche among crevice and bivalve shells. Among the advantages using these assemblages there is the simplicity of sampling. Moreover these species have limited or any mobility, are often very sensible to the pollution and are not affected by the nearby sediment characteristics, but directly respond to the water quality.

Table 1 - Main abiotic variables that could be considered in transitional waters in order to define habitat typologies (after Gibson et al., 2000).

Flow and hydrography	Circulation Tidal regime
Geophysical environment	Soft bottom substrates Hard bottom substrates Beaches Sand flats Mudflats Emergent marshes
Water column characteristics	Salinity Temperature Dissolved oxygen pH Turbidity Nutrients Contaminants Depth
Bottom characteristics	Sediment grain size Total organic carbon Total volatile solids Acid volatile sulphides Sediment redox potential Sediment contamination

Recently a fuzzy logic model to recognise ecological sectors in the lagoon of Venice based also on the benthic sessile community was proposed (Marchini and Marchini, 2006). Meiobenthos includes species with very short life span, therefore could be useful as early warning and short-term effects monitoring, while mainly disadvantages are represented by handle and taxonomic difficulty.

1.4 Habitat typology

In order to monitor, manage and protect transitional water environments, identification of the habitat typologies, delineation of their boundaries and characterisation of the communities that they host, within a consistent classification, is required (Roff and Taylor, 2000). Habitat typology could be defined according to geophysical features (Roff and Taylor, 2000; Roff *et al.*, 2003). The main abiotic variables that could be considered are summarised in Table 1. Geophysical properties and structural biotic components, like seagrasses, should be chosen as determinant factors. In the choice should be avoided redundancy and autocorrelation between variables. The chosen variables can be arranged in hierarchical sequence or combinatory way starting from those that show the greatest ability to discriminate among habitat types (Roff *et al.*, 2003).

Habitat typologies were directly or indirectly considered in several biotic indices. The "Lesina Bioindex" for example is starting from the conceptual scheme of degree of confinement and the relative extension of the so-called "paralic" zones in the Mediterranean coastal lagoons (Guelorget and Perthuisot, 1992). Most of the north American benthic indices of biotic integrity (B-IBI family) considered different metrics (Weisberg *et al.*, 1997) and/or provided separated thresholds (Van Dolah *et al.*, 1999; Eaton, 2001) according to habitat typologies, previously defined on their salinity and/or sediment mud content. Other indices incorporate the salinity directly in the calculation formula (Engle *et al.*, 1994; Engle and Summers, 1999; Paul *et al.*, 2001). In the TWReferenceNET EU project (Management and Sustainable Development of Protected Transitional Waters, EU INTERREG III B 2000-2006, CADSES project 3B073) was adopted a two level factorial classification of habitats, which includes substratum type and prevalent vegetation (Table 2). This combinatory classification defines 12 typologies, although some of them are fairly rare in transitional ecosystems. The most common habitat typology in the European transitional waters ecosystems is mud without vegetation, followed by mud with different kind of vegetation (Table 3).

Table 2 - Habitat typology adopted in the TWReferenceNET EU project and their numeric code.

		Prevalent substratum		
		Rock	**Sand**	**Mud**
Dominant vegetation	**without vegetation**	1	5	9
	macroalgae	2	6	10
	submerged macrophytes	3	7	11
	emergent macrophytes	4	8	12

2 Methodological approaches

Biological monitoring and ecosystem health assessment are based on the possible responses of biotic elements to the different source of perturbation. The biological responses could be analyses at different level of biological organisation ranging from intra organism (biomarkers), single individuals or experimental populations (ecotoxicology) to communities (Attrill and Depledge, 1997).

Table 3 - Habitat typologies found in the study sites considered in the TWReferenceNET (numbers represent the code of Table 2).

		Rock				Sand				Mud			
Transitional Water system	**Geographic area**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Grado lagoon (Italy)	N. Adriatic Sea									X			
Grado Cavanata (Italy)	N. Adriatic Sea									X			X
Grado fish farm (Italy)	N. Adriatic Sea									X			
Pialassa Baiona (Italy)	N. Adriatic Sea									X			
Karavasta (Albania)	S. Adriatic Sea									X	X		
Narta (Albania)	S. Adriatic Sea									X			
Patok (Albania)	S. Adriatic Sea									X		X	
Alimini (Italy)	S. Adriatic Sea					X			X	X			
Cesine (Italy)	S. Adriatic Sea							X				X	X
Margherita di Savoia (Italy)	S. Adriatic Sea									X	X		
Torre Guaceto (Italy)	S. Adriatic Sea											X	X
Agiasma (Greece)	Aegean Sea										X	X	
Kalloni (Greece)	Aegean Sea									X			
Varna (Bulgaria)	Black Sea					X				X			
Leahova (Romania)	Black Sea									X			X
Sinoe (Romania)	Black Sea					X				X			X

It is widely accepted that there is a tiered cascade in biological responses to increasing anthropogenic disturbance. The first responses to stress will occur at low levels of biological organization but if the stress persists or the severity increases, these molecular level effects will lead to cellular alterations followed by tissue and organs dysfunction. Individual metabolic alteration will reflect on population dynamics (e.g., reproduction, recruitment and mortality) and structure, up to local extinctions. Population changes will result in alteration of interspecific relationships at community level, leading ultimately to changes in the functional integrity of ecosystems (Fig. 2; Spurgeon *et al.*, 2005 and references therein). Investigation at the community level is generally considered most ecologically relevant, because involve a wide taxonomic range, reflect the effect of many processes at lower level of biological organization, integrate these processes over a relatively long-term period (Warwick, 1993; Attrill and Depledge, 1997). In contrast studies at lower biological organisation, like biomarkers, could be useful as early warning approach. Biotic indices are classification tools relate to assemblages' organisation. They are often based on species composition and density, but sometimes they consider other characteristic of the assemblages like biomass, taxonomic diversity, trophic guilds, life strategies, size spectra, etc. Some biotic indices integrate several metrics representing different aspect of community organisation and functioning. In most cases they explicitly include sensitivity or tolerance of the species towards pollution or other disturb sources (Hilsenhoff, 1987; Majeed, 1987; Clements *et al.*, 1992; Zamoramunoz and Albatercedor, 1996; Roberts *et al.*, 1998; Borja *et al.*, 2000).

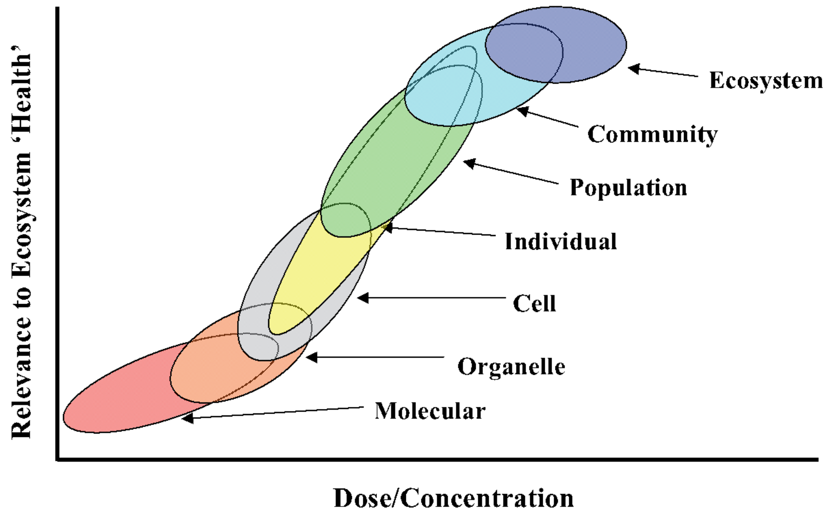

Fig. 2. Schematic diagram of the hierarchical relationship between ecotoxicological responses measured at different levels of biological organization (after Spurgeon et al., 2005).

Species patterns across pollution/disturbance gradients are also explained from species-specific competition abilities under abundant resource conditions (Orfanidis *et al.*, 2001, 2003).

2.1 Species diversity

Species diversity represents an emergent property of the communities (Begon *et al.*, 1986). Species richness and diversity indices were introduced in ecology at the end of 40's (Simpson, 1949; Shannon and Weaver, 1949; Margalef, 1958; Menhinick, 1964; McIntosh, 1967). Afterward several authors developed new indices and discussed their application in different habitats (for review see Washington, 1984; Gray, 2000; Magurran, 2004). Biological diversity refer to the variability among living organisms, including diversity within species, between species and of ecosystems (Magurran, 2004). Species diversity can be investigated at different spatial and temporal scales, which lead different definition (Whittaker, 1972):

α diversity: the diversity of spatially defined units (defined assemblages or within habitat);

β diversity: differences in the compositional diversity in space and time, reflecting biotic change or species replacement, between different areas and/or habitats (e.g. along transects; Whittaker, 1960), different spatial configuration of sampling units (i.e. increasing spatial scale), or changes overtime (i.e. turnover);

γ diversity: diversity of a landscape;

ε diversity: diversity of a biogeographic province;

δ diversity: is defined as the change in species composition and abundances that occurs between units of γ diversity within an area of ε diversity.

Although other terminology was recently proposed (Gray, 2000), these terms were still now in use (see also table 4).

Generally in monitoring and biological assessment programs species diversity was intended as α diversity. Species diversity can be partitioned into two components: richness and evenness (Simpson, 1949). There are several species diversity indices that express one or both of these two components. The indices that combine both aspects in a single

Table 4 - Category of species diversity (Magurran, 2004, after Whittaker, 1972).

Scale	inventory diversity	differentiation diversity
within sample	point diversity	
between sample, within habitat		pattern diversity
within habitat	α diversity	
between habitat, within landscape		β diversity
within landscape	γ diversity	
between landscape		δ diversity
within biogeographical province	ε diversity	

statistic are often indicated as "heterogeneity indices".Although most of the ecological studies carried out in transitional water consider species diversity, absolute reference values suitable to define the status of assemblages inhabiting these habitats doesn't exist. Several multimetric benthic biotic indices incorporate one or more measures of species diversity (see 4.11). In these cases relative threshold values were comparatively defined according to local reference conditions.

2.2 Pearson and Rosenberg model

Sediment organic enrichment was considered one of the most common disturbances that affect benthic assemblages. Relationships between benthic community characteristic and level of organic enrichment were described by the widely accepted Pearson and Rosenberg model (Pearson and Rosenberg, 1978). According to this model, along a gradient of increase organic contents species richness decrease, numbers of individual increase, as a results of high density of few opportunistic species, overall biomass decrease, except for a small increase at the peak of opportunists, and generally average body size decrease (Fig. 3).

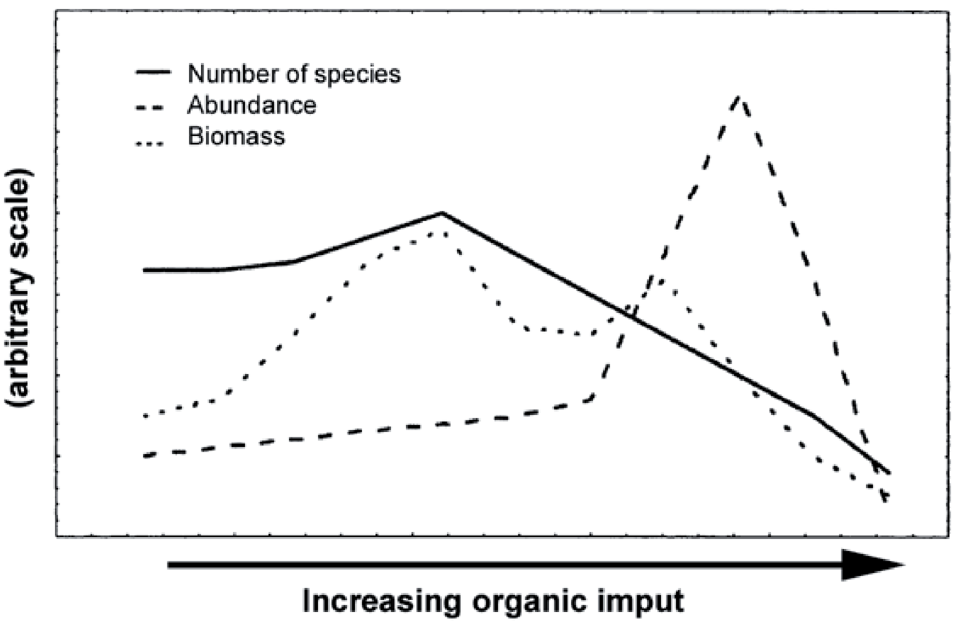

Figure 3. SAB (Species, Abundance and Biomass) graphical model proposed by Pearson and Rosenberg (1978) (after Gray et al., 2002).

Changes described by Pearson and Rosenberg does not concern just species richness, abundance and biomass, but also the composition of the species assemblage with an increase of species tolerant to hypoxic conditions (Gray, 1979; Diaz and Rosenberg, 1995; Gray *et al.*, 2002; Hyland *et al.*, 2005). Based on this model several methods and indices have been proposed in order to assess the environmental quality of coastal areas, including transitional waters. Some of them consider only synthetic descriptor like species richness and evenness, while other take into account only species composition (Grall and Glémarec, 1997; Majeed, 1987; Borja *et al.*, 2000).

Should be note that organic enrichment could be due to both anthropogenic sources and natural reason, like local hydrodynamic condition that increase sedimentation rate or reduce water turnover (Gray *et al.*, 2002). Whatever is the origin of the organic enrichment, it leads to oxygen depletion and build-up toxic products (ammonia and sulphide) associated to the decomposition processes (Viaroli *et al.*, 2004; Hyland *et al.*, 2005). Hydrology plays major roles in establish and maintain hypoxic and dystrophic conditions. The main effects on benthic fauna result from hypoxia and toxicity of sulphide rather than organic enrichment per se (Gray *et al.*, 2002).

Biotic indices based solely on Pearson and Rosenberg model frequently assumes that is possible to transpose this general trend to any kind of pollution or disturbance.

2.3 Indicator taxa

Several authors attributes to single "focal" species an important role in monitoring programs, and in conservation and managements projects (for a review see Zacharias and Roff, 2001 and references therein). Among focal species it is possible distinguish between:

- "flagship" species, which have not particularly echo-functional or indicator role but could be used as a tools to garner public support, in transitional waters could be represented for example by some fishes;
- "umbrella" species, which are those whose conservation will also preserve other species, like seagrasses;
- "keystone" species, which are critical to the ecological function of a community or habitat, the importance of these species is disproportionate to their abundance or biomass, like some top predators or seagrasses;
- "indicator" species, which are taxa whose presence (or absence) denotes either the composition or condition of a particular habitat, community, or ecosystem.

These definitions are not exclusive and same species could be fall in more than one category. In the ecosystem quality assessment, indicator species play a major role. If an organism known to be intolerant of pollution is found to be abundant at a site, high water quality conditions can be inferred. On the other hand, dominance by pollution tolerant organisms could imply a degraded condition. When available, indicator taxa could be an important, cost-effective preliminary survey tool for site assessments. However, ecosystem health assessment should be never based only on some indicator species.

Among indicator species that could be monitored in transitional waters there are several amphipods that are known sensitive to some pollutants. In San Francisco Bay, for example, was demonstrated the sensitivity of the amphipod *Rhepoxynius abronius* to a complex contaminant mixture including pesticides (Swartz *et al.*, 1994).

Much more questionable is the attribution of the roles as indicator of degraded systems to tolerant and so called "opportunistic" species. The relation of their presence and abundances with the environmental conditions are often speculative and not really proved. Typical study case is represented by the polychaete *Capitella capitata* (actually a complex of sibling species; Grassle and Grassle, 1976; Gamenick *et al.*, 1998). Often, the presence of this indicator species corresponds to a dominance of deposit feeders that colonize an area as organic pollution increases. A problem with using pollution tolerant indicator organisms is that some of these organisms may be ubiquitous and found in naturally occurring organically enriched habitats as well as in minimally impaired ecosystems. Tolerant and ubiquitous organisms can be found in sediments far away from sources of disturbances. The use of the concept of "clean" indicator species is less subject to this form of misinterpretation. These "clean" or highly sensitive organisms are less likely to be found in both polluted and high quality habitats.

Several biotic indices, in some way, used taxa as indicator attributing some ecological importance to each species that compose the assemblages. The progenitor of such indices could be considered Hilsenhoff, which first use arthropods to evaluate water quality in freshwaters (Hilsenhoff, 1977; Hilsenhoff, 1987). Afterward several similar indices were proposed for freshwater ecosystems (Lenat, 1993; Hawkes, 1998; Graça and Coimbra, 1998; Spaggiari and Franceschini, 2000). Among the benthic biotic indices for coastal and transitional waters, BRI (Smith *et al.*, 2001), AMBI (Borja *et al.*, 2000) and BENTIX (Simboura and Zenetos, 2002) are clear examples of this approach. But practically every biotic indices of integrity include a measure of the relative abundance of species (or higher taxonomic groups) considered sensitive or tolerant toward pollution or other disturbs.

Although AMBI provide ecological group assignment for more than three thousands taxa, most of them are based only on "expert judgments" while experimental data on pollution sensitivity are available only for a limited number of species. To overcome this issue, biotic indices are often calculated only on a little subset of the whole species list, choosing the species on which sensitivity data are available. Attempts to generalize species sensitivity to higher taxonomic levels (e.g. genus or family) were also made (Van Dolah *et al.*, 1999). Although it is possible to some extent to recognize a general trend of response to stress even at phylum or class level (e.g. in order of rising sensitivity to oxygen depletion: bivalves, annelids, crustaceans, fishes; for a review see Gray *et al.*, 2002), sensitivity can also change dramatically within the same genus or family. Therefore, any generalization on sensitivity should be made carefully.

2.4 Taxonomic resolution

Several authors investigated the level of taxonomic resolution (i.e. genera, family, order, class and phylum) needed to detect pollution effects (Ellis, 1985; Warwick, 1988; James *et al.*, 1995; Somerfield and Clarke, 1995; Olsgard *et al.*, 1997; Olsgard *et al.*, 1998). They generally indicate that identification of organisms to the lowest possible taxon may not always be necessary to enable description of spatial patterns in routine pollution monitoring programs. Often family level can represent an acceptable approximation for descriptive and comparative studies on macrobenthic assemblages (Somerfield and Clarke, 1995; Olsgard *et al.*, 1998). Even higher taxonomic level (i.e. order and class) could be enough in multivariate analyses of benthic assemblages in lagoonal ecosystem (Mistri and Rossi, 2001). However, when biotic indices based on species sensitivities are applied to assemblages' data, aggregation to any higher taxonomic level should be avoid, unless specifically foreseen for some groups (Borja and Muxika, 2005).

2.5 Ecological strategies and functional diversity

A community "well structured" that includes species representing a wide range of ecological strategies is generally considered expression of pristine habitats. Any measure of departure from balanced condition could be theoretically adopted as biological index of ecosystem health. Starting from this assumption, several studies were carried out comparing communities in term of feeding and/or reproductive strategies.

Undisturbed communities tend to be characterised by few large and long life span species represented by rather few individuals, which are in equilibrium with the available resources. These species are frequently called conservative or *K* selected species. Conversely, assemblages found in disturbed habitats are dominated by small and short life-span species, also called opportunist or *r*-selected species, represented by large numbers of individuals. From this assumption Worwick proposed to assess the community health comparing the *k*-dominance curves (Lambshead *et al.*, 1983) for both species abundance and biomass (ABC method; Warwick, 1986). Following this method a high biomass curve, overhanging the abundance curve, indicate an undisturbed community, while abundance curve higher than biomass should be a symptom of disturbed community (Fig. 4).

Functional diversity concern the number, type and distribution of functions performed by organisms within an ecosystem (Diaz and Cabido, 2001). Several biological traits could be considered, like feeding behaviour, mobility, etc. (Bremner *et al.*, 2003). Some trophic indices based on feeding strategies were already developed (Word, 1978; Gaston and Nasci, 1988; Paiva, 1993; Rizzo *et al.*, 1996; Pinedo *et al.*, 1997). Other approaches to the functional diversity could be represented by the trophic group analysis and biological trait analysis developed for terrestrial plant and freshwater invertebrates (Bremner *et al.*, 2003; Bady *et al.*, 2005).

Main disadvantage come from the difficult to assess the real diet of the organism, since is often impossible or time-consuming observing the stomach content. Assign trophic guild to each taxon is sometime aleatory because several species change feeding behaviour during the life or even adapt their diet according to environmental condition and food availability (Fauchald and Jumars, 1979). Those facts nowadays lead to doubt about the possibility of a clear separation among trophic guilds (Jørgensen *et al.*, 2005b).

2.6 Body size descriptors

The advances in searching for simple and

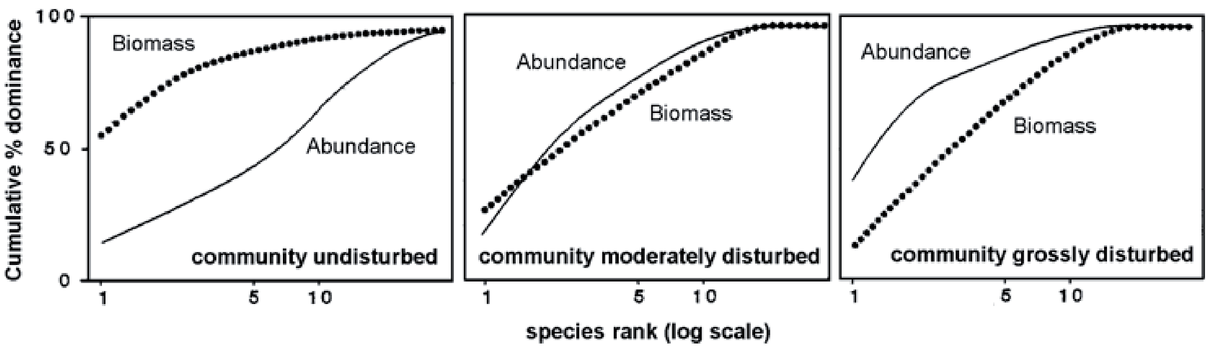

Figure 4. Theoretical k-dominance curves for species abundance and biomass comparison (ABC) for undisturbed, moderately disturbed and grossly disturbed communities (after Warwick, 1986).

effective descriptors of biological ecosystem health lead to consider functional related descriptors independent to taxonomic composition of the assemblages. Body-size-abundance distributions and biomass-size spectra are structural features of aquatic communities (Sheldon *et al.*, 1972; Sprules and Munawar, 1986; Sprules *et al.*, 1991; Bourassa and Morin, 1995; Morin *et al.*, 2001).

Body size relates body-size-abundance distributions to disturbance pressures through individual energetics, population dynamics, interspecific interactions and species coexistence responses (Basset, 1995; Basset *et al.*, 2004). Main advantages of body size and related descriptors are:

- body-size-abundance distributions are consistently less variable than taxonomic composition;
- the width of body-size-abundance distribution is mainly due to the interspecific component;
- the descriptors of body-size-abundance distributions seem to respond on environmental gradients and generally co-vary with species density, richness and diversity (Basset *et al.*, 2004; Sabetta *et al.*, 2005).

Body size is generally easy to measure, and body-size-related descriptors can undergo intercalibration procedures, which is a crucial requirement of monitoring programmes that is not always fulfilled by descriptors of biological quality elements related to the taxonomic component. As disadvantage, a description of the size–abundance distribution requires large samples of individuals, and obtaining such data can be time consuming. Although biotic indices based on body-size-related descriptors applied to both plankton and benthos guilds promise to become effective tools for environmental quality assessment, at present more data are required in order to evaluate the actual response of these descriptors to external perturbations. Moreover methodological standardization is also required in order to reduce variability of responses, enhance adequacy of intercalibration and translation of these responses into an ecosystem health index.

3 Classification of indices

3.1 Single metrics vs. multimetric indices

A metric is a calculated term or enumeration representing some aspect of biological assemblage structure, function, or other measurable characteristic, which can be expressed numerically as integers or ratios. Single metrics values can be used as indicator and compared directly to the reference condition, without development of an index. This simplified approach could be justifiable where there are practical constrain like, for instance, using as reference some paleoecological data.

The multimetric approach consist in an array of metrics or measures that individually provide limited information on biological status, but when integrated, act as an overall indicator of biological condition. Metrics incorporate information from individual, population, and community levels into a single, ecologically-based index of aquatic ecosystem health. One of the first example of multimetric index was the fish Index of Biotic Integrity (IBI; Karr, 1981; Karr *et al.*, 1986), which aggregates various elements and surrogate measures of process into a single assessment of biological condition. Developed initially for streams (Karr, 1981; Karr *et al.*, 1986; Seegert, 2000a; Seegert, 2000b), the multimetric approach has increasingly been applied to transitional waters ecosystem, starting from north American estuaries (Weisberg *et al.*, 1997; Hyland *et al.*, 2000; Eaton, 2001). The multimetric indices are typically a sum or an average of standardized scores of its component metrics. Develop of this indices staring from the comparison and selection of metrics that differ between reference

Table 5 - Potential metrics for macrophytes, benthic macroinvertebrates, and fish that could be considered for estuaries (from Gibson *et al.* 2000).

	Richness	Composition	Tolerance	Trophic/Habitat
Macrophytes	▸ Not applicable	▸ Not applicable	▸ TSS ▸ light attenuation ▸ Chlorophyll *a* ▸ DIN ▸ DIP	▸ % cover ▸ density of new shoots ▸ biomass ▸ stem counts
Benthic Macroinvertebrates	▸ dominant taxa ▸ taxa richness ▸ Shannon-Wiener Diversity Index ▸ mean # of species ▸ Pielou's Evenness Index	▸ # amphipods per event ▸ amphipod biomass ▸ mean abundance of bivalves/site ▸ # of gastropods per event	▸ % polychaetes ▸ polychaete biomass	▸ % or biomass epibenthic ▸ % or biomass deposit feeders ▸ % or biomass suspension feeders
Fish	▸ dominant taxa ▸ taxa richness ▸ # of estuarine spawners ▸ # anadronomous spawners ▸ total fish exclusive of Atlantic menhaden	▸ total # of species ▸ # species in bottom trawl ▸ # species comprising 90% of individuals	▸ #, % or biomass of menhaden	▸ Proportion of planktivores ▸ Proportion of benthic feeders ▸ Proportion of piscivores

and degraded sites. Then thresholds and standardised scores were defined for each metric in order to combine the scores in a single index. A comparison of the main metrics measurable for different biological quality elements are given in table 5, while a list of possible macrobenthic invertebrates metrics with possible response to disturbs events is summarised in Table 6. Most of the multimetric indices include a measure of the relative abundance of some “pollution-indicative” and/or “pollution-sensitive” taxa.

3.2 Discriminant analysis and multivariate ordination

Combining different metrics in a biotic index require standardizing and/or scaling the values that each metrics can assume and find the correspondence and departure of the different combination from the reference conditions. A way to distinguish reference conditions from impaired states is using the multivariate discriminant analysis model. The calibrated model is then applied to assessment sites to determine whether they are impaired. This approach was applied to northern Gulf of Mexico estuaries (Engle *et al.*, 1994; Engle and Summers, 1999) and Virginian Biogeographic Province (Paul *et al.*, 2001).

Multivariate ordination approaches were applied to examine differences in species composition between reference and impaired sites. Examples of these approaches were represented by the study of the effects of oil drilling in the North Sea (Warwick and Clarke, 1991), and the index developed for benthic quality in California (Smith *et al.*, 2001).

3.3 Taxonomic vs. not taxonomic descriptors and indices

Assemblages can be described and analysed in term of species composition and biomass but also in term of trophic and functioning structures. All the descriptor and biotic

Table 6 - Potential metrics for benthic macroinvertebrates that could be considered for transitional waters (from Gibson *et al.* 2000).

Metric	Response to disturb
No. of taxa	reduced
Mean no. of individuals per taxon	substantially lower or higher
% contribution of dominant taxon	elevated
Shannon diversity	reduced
Total biomass	substantially lower or higher
% biomass of opportunistic species	elevated
% abundance of opportunistic species	elevated
Equilibrium species biomass	reduced
Equilibrium species abundance	reduced
% taxa below 5-cm	reduced
% biomass below 5-cm	reduced
% carnivores and omnivores	elevated
No. of amphipod species	reduced
% individuals as amphipods	reduced
% individuals as polychaetes/oligochaetes	elevated
No. of bivalve species	reduced
% individuals as molluscs	reduced
% individuals as deposit feeders	elevated
Mean size of organism in habitat	reduced
Proportion of expected no. of species in sample	reduced
Proportion of expected no. of species at site	reduced
Mean weight per individual polychaete	reduced
No. of suspension feeders	reduced
% individuals as suspension feeders	reduced
No. of gastropod species	reduced
No. of Capitellid polychaete species	elevated

indices that explicitly or implicitly take into account the single species composing the assemblages could be defined as "taxonomic". They required the identification of all the specimens at the lowest possible (or practicable) taxonomic level. Sometimes it is sufficient enumerate the taxa and obtain the abundance and/or biomass for each of them, even without knowing the exact name of each species (e.g. diversity indices).The "not taxonomic" descriptors and indices were based on ecological approaches that overcome the necessity of the taxonomic identification. They include ecotoxicological, functional, size and biomass descriptors. There are also mixed taxonomic and not taxonomic indices. A scheme of classification is reported in table 7.

4 Benthic macroinvertebrate

4.1 Species diversity indices

Species diversity indices can be applied in order to compare the assemblages within and between transitional water systems. Major requirements are the application of the same sampling methods (in term of sampling device, area and/or volume, number of replicates and individual size range, i.e. sieve mesh size) and taxonomic resolution.

To compare studies carried out by different researchers in different place or time, standard protocol for data collection and laboratory analyses is of paramount importance.

Although several multimetric benthic biotic indices incorporate one or more measures of species diversity (see 4.11), reference values and interpretative thresholds vary according to habitat typologies and local reference conditions.

4.1.1 Species richness

The most common and simple expression of species richness is the mean number of species per sampling area (S). Since species richness estimates depend on sampling effort, some authors proposed to compensate the sampling effects by dividing the number of species recorded by the sample dimension in terms of total number of individuals (N) in the sample. The most well known formulas are:

Margalef's species richness

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

and Menhinick's species richness

$$D_{Mg} = \frac{S-1}{\ln N}$$

Thought the compensation attempts, these indices remain affected by sample size. Species richness of a given habitat could be estimated applying some models, like species accumulation and species rarefaction curves, as well as other parametric and nonparametric methods (for more details see Krebs, 1989 and Magurran, 2004). For comparative purpose, when a standard sampling protocol was adopted, S could be considered the most effective expression of species richness.

4.1.2 Heterogeneity

Overall heterogeneity measure can be obtained through several diversity indices. The most common are:

Simpson's diversity index (Simpson, 1949):

$$D = \sum \left(p_i^2\right)$$

where p_i is the proportion of individuals found in the ith species.

Since as D increase, diversity decrease, this formula is often called Simpson's "dominance" index. Otherwise the following transformation were adopted: 1-D, 1/D, -ln(D).

Shannon's diversity index (Shannon and Weaver, 1949):

$$H' = -\sum \left(p_i \cdot \log p_i\right)$$

Although Shannon's index is often calculated using the $\log_2$ for historical reason, in ecology

Table 7 - Possible classification of indices

Typology	Group	Indicator or Index	Biological Elements	References
Taxonomic	Species richness	*S*	A	Margalef, 1958
		d	A	Margalef, 1958
	Diversity indices	*H'*	A	Shannon and Weaver, 1949
		D	A	Simpson, 1949
		J	A	Pielou, 1966
		Δ+, Λ+	A	Warwick and Clarke, 1995
	Biotic indices	*AMBI*	B	Borja *et al.*, 2000
		BENTIX	B	Simboura and Zenetos, 2002
		IBI	F	Karr, 1981
		EBI	F	Hughes *et al.*, 2002
		B-IBI	B	Engle *et al.*, 1994; Weisberg *et al.*, 1997; Engle and Summers, 1999; Van Dolah *et al.*, 1999; Eaton, 2001; Paul *et al.*, 2001; Thompson and Lowe, 2004
		Bioindex LESINA	B	Breber, 1997, Breber *et al.*, 2001
		SWAMPS	B	Chessman *et al.*, 2002
		(*R/C*), (*C/Ph*)	M	Sfriso *et al.*, 2002; Fano *et al.*, 2003
Mixed		*EQI*	B	Fano *et al.*, 2003
		FINE	B	Mistri *et al.*, 2005
Not taxonomic	Functional	Functional diversity	B	Bremner *et al.*, 2003; Bady *et al.*, 2005
		EEI	M	Orfanidis *et al.*, 2001, 2003
	Size	*BSS*	B, P	Reizopoulou *et al.*, 1996; Lardicci and Rossi, 1998, Basset *et al.*, 2004
		ISD	B	Reizopoulou and Nicolaidou. A., 2004

natural logs was sometimes preferred even if there are no pressing biological reasons to prefer one base instead of another, therefore the logarithm base applied should be always declared. Although superiorly unlimited, in practice Shannon's index, whatever base was applied, tend to assume values in a very narrow interval, which lead sometimes in difficult in distinguish differences among sites. To meaningful the measure often the index was transformed by ExpH'(where Exp is the base of logarithm applied). This transformation, also known as Hill's N1 index, intuitively gives the number of species that would have been found in the sample had all species been equally common (Whittaker, 1972; Hill, 1973).

Although Shannon's index was widely applied, a general agreement on the relationship of the index and the ecological status is far to achieve. A possible interpretation scale proposed for Norwegian fjords, using H' ($\log_2$) is (Molvær *et al.*, 1997):

0-1 bad status
1-2 poor status
2-3 moderate status
3-4 good status
> 4 high status

4.1.3 Evenness

Both Simpson and Shannon's indices are expression of the overall heterogeneity, the corresponding evenness component can be obtained dividing the values by the maximum diversity that could be possible occur, which is the situation where all species have equal abundances. For the Shannon's index, the evenness component is the:

Pielou's index:

$$J' = H' / \log S$$

(using the appropriate base of logarithm)

While for the N1 index, the corresponding evenness component, often-called Hill's N10 index, is calculated by N1/S (Hill, 1973).

4.1.4 Phylogenetic and functional diversity

Species richness, evenness and overall heterogeneity take into account only the number of species and their abundances (or biomass). These indices treat all species as equal, in practice it is sufficient to enumerate the taxa; no knowledge about their biological, ecological or phylogenetic characteristics was required. From a biological evolution and ecological point of view, an assemblages constituted by species genetically or evolutionary very distant and/or functionally different, could be considered more diversified compared to an assemblages with the same number of species and relative abundances but genetically or functionally homogeneous. The first attempt to consider the differences between the species and their singleness was made adapting the Shannon's index on taxonomical bases (i.e. including familial, generic and species diversity; Pielou, 1975). A simple estimation of phylogenetic diversity can be obtained summing the branch length within a taxonomic tree (PD index; Faith, 1992). Clarke and Warwick introduced a taxonomic distinctness measure, which is a natural extension of Simpson's index (Warwick and Clarke, 1995; Clarke and Warwick, 1998; Warwick and Clarke, 1998). These measures consider the path length in a Linnaean taxonomic tree between two randomly chosen organisms. There are two forms: considering both species abundances and taxonomic relatedness:

Taxonomic diversity index:

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

or considering only the taxonomic relatedness:

Taxonomic distinctness index:

$$\Delta^* = \frac{\sum\sum_{i<j} \omega_{ij} x_i x_j}{\sum_{i<j} x_i x_j}$$

where ωij is the taxonomic path length between two species, x_i and x_j are the abundances of the ith or jth species.

When presence/absence data are used both measures reduce to the same statistic:

$$\Delta^{+} = 2\frac{\sum\sum_{i<j}\omega_{ij}}{S(S-1)}$$

where S is the number of species in the study.

The corresponding evenness of the distribution of taxa across the hierarchical taxonomic tree is (Clarke and Warwick, 2001 Warwick and Clarke, 2001) the variation in average taxonomic distinctness index:

$$\Lambda^{+} = \frac{\sum\sum_{i\neq j}\omega_{ij}^{2}}{S(S-1)} - \overline{\omega}^{2}$$

where

$$\overline{\omega} = \frac{\sum\sum_{i\neq j}\omega_{ij}}{S(S-1)} \equiv \Delta^{+}$$

Phylogenetic measurement of diversity could be calculated on the base of genetic distances. Although genetic distances have much more biological significance then an artificial taxonomic hierarchical tree, they are far to be available for all pair of species. Following the taxonomic diversity approach, functional diversity can be calculated on the base of the total "branch length" of a dendrogram constructed from species trait values, which are linked to the ecosystem process of interest (FD index; Petchey and Gaston, 2002a; Petchey and Gaston, 2002b).

Although diversity indices, as well as phylogenetic and functional diversity indices were applied in several monitoring and quality assessment studies carried out in transitional water ecosystems, still now no reference values to distinguish between impaired and pristine sites were proposed both at local or regional scale. Comparing three coastal lagoons at different level of human impact and eutrophication in the south of French (Mediterranean Sea), the number of macrobenthic species found with the same sampling effort ranging from 7 in the most impacted ecosystem to 27 in the less impacted (Mouillot *et al.*, 2005b). In this study, the average taxonomic distinctness (Δ+) was inversely related to eutrophication level, while variation in taxonomic distinctness (Λ+) increased with eutrophication.

Diversity indices, including taxonomic distinctness, were used to investigate benthic communities along the land-seaward gradient in two dates (year 1976 and 1989) in Sacca degli Scardovari, a northern Italian Adriatic coastal lagoon (Mistri *et al.*, 2000). Although multivariate ordination (nMDS) showed clear pattern between date and along the environmental gradient, the univariate indices were able to clearly describe the gradient only in the second sampling date.

4.2 ABC method and W-statistic index

Starting from the graphic ABC methods (Warwick, 1986), based on the comparison of the abundance and biomass k dominance curve, Clarke derived the W-statistic index (Clarke, 1990):

$$W = \Sigma^{S}_{i=1} (B_i - A_i) / [50 (S - 1)]$$

where S is the number of species and A_i and B_i are the abundance and biomass respectively of the ith species. W assumes values between +1, indicating undisturbed system, and -1, extremely polluted. Values close to 0 indicate a moderate level of disturb.

The method was demonstrated robust in a wide range of situation, even in some Mediterranean lagoons (Reizopoulou *et al.*, 1996). On the contrary, some studies obtained confusing results applying the W-statistic to macrobenthic assemblages in transitional water ecosystem (Beukema, 1988; Lardicci and Rossi, 1998). That probably because in such ecosystem persist a natural disturb and there are many species that could confound the ABC curves (Lardicci and Rossi, 1998).

4.3 “Lesina” Bioindex

Breber and colleagues proposed the “Lesina” Bioindex as a tool for “ecological quality” assessment of Italian coastal lagoon, in order to answer the requirement of the Italian law D. Lgs.152/99 concerning the preservation of land and sea waters (Breber, 1997; Breber *et al.*, 2001). This index is based on the concept of indicator species associated to the oxygen and salinity conditions, following the conceptual scheme of confinement (Guelorget and Perthuisot, 1983; Guelorget and Perthuisot, 1992). According to the confinement scheme, within the Mediterranean coastal lagoons could be distinguished six “paralic” or “confinement” zones along the confinement gradient, from zone I which is the most marine-influenced to zone VI which is the most confined and where biological populations are different depending on the water balance of the basin (Fig. 5). These zones should be recognized on bionomic bases, according to living species (Table 8). The relative extent of the zones should define the “ecological quality” of the

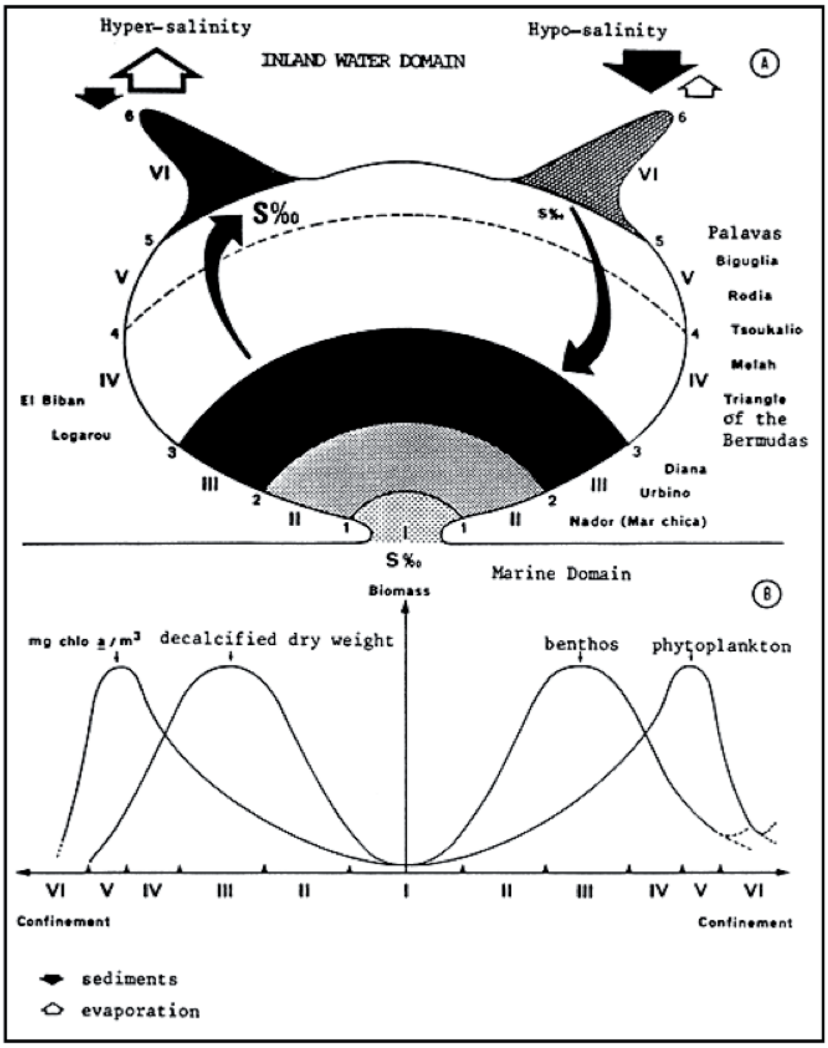

Figure 5. Biological zones defining the degree of confinement in the Mediterranean model of the paralic ecosystem. Quantities of benthos and phytoplankton in relation to degree of confinement were also reported (after Guelorget and Perthuisot, 1983).

lagoon.
The authors suggested to sample the macrobenthic invertebrate assemblages following a stratify design dividing the lagoon in 50 or more sub areas, taking a sample unit for each sub area. They also suggest to sample in autumn, after the eventually dystrophic crises, in order to evaluate the worst situation. Proposed methods include 15x15 cm sampling area and 1 mm sieve size. Each sample unit must be attributed to one of the six bionomic zone according to the species found (Breber *et al.*, 2001). The formula of the index is:

$$\Lambda = \ln\left(\sum_{i=1}^{6} \frac{n_i}{N} \cdot b_i \cdot S_i\right)$$

where i indicate the paralic zone (I-VI) , n_i the number of sample units falling in each zone, N the total number of sample units, b_i the mean biomass in grams of wet weight for the ith zone, S_i the number of species. The index should assume values between 1, worst conditions, and 10, higher ecological quality. The index is intended to evaluate the overall

Table 8 - Species characterising the paralic zones (according to Breber et al., 2001)

Zone			
I	**Bivalves**	*Pharus legumen*	**Crustaceans**
	Spisula subtruncata	*Ensis minor*	*Iphinoe trispinosa*
	Glycimeris insubrica	*Solen marginatus*	
	Acanthocardia tuberculata		**Polychaetes**
	Donax venustus	**Gastropods**	*Nephtys hombergii*
	Tellina pulchella	*Nassarius pygmaeus*	*Sigalion mathildae*
	Tellina planata	*Bela nebula*	
	Tellina fabula	*Acteon tornatilis*	**Echinoderms**
	Mactra stultorum	*Neverita josephinia*	*Echinocardium mediterraneum*
	Scrobicularia cottardi	*Nassarius mutabilis*	
II	**Bivalves**	**Polychaetes**	**Echinoderms**
	Mactra corallina	*Cirriformia tentaculata*	*Asterina gibbosa*
	Mactra glauca	*Magelona papillicornis*	*Holothuria poli*
	Tellina tenuis	*Owenia fusiformis*	*Paracentrotus lividus*
	Donax semistriatus	*Phyllodoce mucosa*	
	Donax trunculus	*Pectinaria koreni*	
	Acanthocardia echinata		
	Dosinia exoleta		
III	**Bivalves**	**Gastropods**	**Polychaetes**
	Tapes decussatus	*Akera bullata*	*Nephtys hombergii*
	Tapes philippinarum		*Armandia cirrhosa*
	Paphia aurea	**Crustaceans**	*Glycera convoluta*
	Scrobicularia plana	*Upogebia pusilla*	
	Corbula gibba		
	Loripes lacteus		
	Gastrana fragilis		
	Anadara diluvii		
IV	**Bivalves**	**Gastropods**	**Polychaetes**
	Abra segmentum	*Cyclope neritea*	*Nereis diversicolor*
	Cerastoderma glaucum	*Hydrobia acuta*	*Perinereis cultrifera*
	Mytilaster minimus		
		Crustaceans	
		Corophium insidiosum	
V	**Gastropods**	**Crustaceans**	**Polychaetes**
	Hydrobia acuta	*Corophium insidiosum*	*Nereis diversicolor*
	Pirenella conica		
VI	Sessile and burrowing macrofauna absent		

lagoonal ecosystem, not the inner disturbance gradients. Attribution of sampling units (and sub areas) to different bionomic zones appeared almost subjective. Even the different importance attributed to these zones is questionable.
Although this index could able to detect some long time evolutional trends, like salinity and hydrodynamic changes, increasing eutrophication or organic enrichment, it seems to be little sensitive to chemical pollution. This index was applied only to the south Italian Varano and Lesina coastal lagoons.

4.4 Water quality index for Catalunya wetlands (QUALES)
The water quality index for Catalunya wetlands (QUALES; Boix *et al.*, 2005), was developed on the basis the studies carried out in ponds, lagoons and marshes of the north-eastern Iberian peninsula. This index incorporates a measurement of taxon sensitivity (called ACCO index), and the taxonomic richness of crustaceans and aquatic insects (called RIC index):

QAELS = (ACCO+1) x log(RIC+1)

where ACCO index is calculated from the relative abundance of each microcrustacean taxon (Cladocera, Copepoda and Ostracoda) weighted by an ecological quality requirement coefficient, which was obtained for each taxon by means of partial canonical correspondence analysis; while RIC index is the sum of the number of crustacean genera, plus the number of families of immature stages of insects (nymphs, pupae and larvae), plus the number of adult Coleoptera and Heteroptera genera (for more detail see Boix *et al.*, 2005).
Sampling method required a standard dip-net (mesh size: 250 μm) and a fixed number of sweeps. The method includes different scores and quality coefficients for athalassohaline wetlands (salinity > 5 psu without marine source), thalassohaline wetlands (salinity > 5 psu with marine source), permanent and temporary freshwater wetlands. Moreover, QUAELS index values were assigned to five categories of water quality following the European Water Framework Directive.
The index was developed and applied in 99 shallow lentic ecosystems. Although some transitional waters were considered in this dataset, this index appeared more oriented to freshwaters ecosystems.

4.5 Swan Wetlands Aquatic Macroinvertebrate Pollution Sensitivity (SWAMPS)
Swan Wetlands Aquatic Macroinvertebrate Pollution Sensitivity (SWAMPS) is a taxonomic biotic index proposed by Chessman *et al.* in order to evaluate the health of wetlands near Perth, Western Australia. A score, ranging between 1 and 100, is assigned to each taxon according to its sensitivity to anthropogenic disturbance, primarily nutrients enrichment (Chessman *et al.*, 2002). SWAMPS for individual wetlands were calculated as abundance-weighted or unweighted means of the scores of all taxa present in standard samples. Samples were taken by a D-framed sweep net with a mouth area of 0.034 m2 and 0.25 mm mesh, moved from the water surface to the bottom ten times over a distance of 10 m.Scores can be assigned at species level (SWAMPS-S) or at family level (SWAMPS-F). SWAMPS-S is much more accurate and well related to nutrients enrichment and chemical and physical characteristic of water than SWAMPS-F. A list of 80 families and 246 species from the Western Australia was provided (Chessman *et al.*, 2002). The interpretation of SWAMPS index appropriate for the Western Australia is summarised in table 9.

4.6 Weighted Biotic Index (WBI)
The Weighted Biotic Index (WBI) could be considered a semi-quantitative

Table 9 - Indicative interpretation of SWAMPS-F level and SWAMPS-S level scores for wetlands on the Swan Coastal Plane, Western Australia (Chessman *et al.*, 2002).

Interpretation for the Swan Coastal Plane	***SWAMPS-S***	***SWAMPS-F***
Cultural eutrophication or other human impact is likely	<46	<42
Cultural eutrophication or other human impact may be present (more investigation is needed)	46 - 49	42 - 44
Cultural eutrophication is unlikely (but the possible presence of an unusual human impact to which SWAMPS is not sensitive should not be ignored	>49	>44

rapid assessment method based on the SWAMPS index (Chessman *et al.*, 2002). Macroinvertebrate samples are collected, for each dominant habitat, using the same sweep net (250 μm mesh) but limiting the sampling time to 2 minutes. Samples are sorted 'on-site' while the invertebrates are alive. No more than two hundred animals are picked from the samples, with a maximum of ten individuals of each family or morphotype to be selected. Samples are sorted for a maximum of 30 minutes. Abundances are visually estimate in four classes, which correspond to an abundance score (Table 10), then preserved for further classification to the family level.

The Weighted Biotic Index is calculated as:

$$WBI = \sum_i \left(AS_i \times SGV_i \right) \Big/ \sum_i AS_i$$

where AS_i is the abundance score and SGV_i is the SWAMPS score value at family level (Table 11). Wetlands can be classified into three categories of environmental quality, in the same way of SWAMPS index, on the basis of their average score values (Table 12).

This index was adopted by the National Action Plan for Salinity and Water Quality (NAP) and by the Natural Heritage Trust (NHT) programs at the regional level, which are cooperatively implemented by the Australian, State and Territory Governments. As deducible from the sampling methods described above and the taxa list, both SWAMPS and WBI indices are mainly oriented to freshwaters and low salinity wetlands, whose macroinvertebrate assemblages are characterised by insects and freshwater crustaceans. Moreover sampling method involves also zooplankton species.

Table 10 - Abundances classes

Score	**Class**	**Abundance**
1	Rare	<10 ind./sweep
2	Common	11–100 ind./sweep
3	Abundant	101–1000 ind./sweep
4	Highly abundant	>1000 individuals /sweep

4.7 Ecofuntional Quality Index (EQI)

The Ecofuntional Quality Index (EQI) is a multimetric index for the evaluation of environmental quality in transitional waters developed using biotic data from three Italian coastal lagoons (Fano *et al.*, 2003). This index incorporates measures of primary productivity (as phytoplankton, seaweed and seagrass biomasses), structure and productivity of the benthic community (as numerical abundance, biomass density, number of taxa, and taxonomic diversity of macrozoobenthos), and trophic complexity (expressed as macrozoobenthic functional diversity). The index is obtained by the sum of weights given to these seven or eight metrics (dependent if macrophytes are present or not), each transformed onto a dimensionless 0–100 quality scale. This standardization is simply made by assigning 100 to the highest value fund, and by normalising to 100 all

Table 11 - SWAMPS grades for families of macro invertebrates recorded from wetlands on the Swan Coastal Plain, Western Australia.

Family	Score	Family	Score
Aeshnidae	65	Libellulidae	73
Amphisopidae	31	Limnesiidae	64
Ancylidae	72	Limnetidae	50
Arrenuridae	59	Limnocharidae	66
Baetidae	69	Limnocytheridae	73
Bosminidae	64	Lymnaeidae	58
Caenidae	71	Macrothricidae	79
Candonidae	63	Megapodagronidae	36
Ceinidae	50	Mesoveliidae	58
Centropagidae	100	Moinidae	42
Ceratopogonidae	65	(Nematoda)	46
Chironominae	44	Noteridae	36
Chrysomelidae	65	Notonectidae	46
Chydoridae	38	(Oligochaeta)	55
Coenagrionidae	47	Oribatidae	70
Corduliidae	72	Orthocladinae	46
Corixidae	6	Oxidae	63
Culicidae	66	Palaemonidae	83
Cyclopidae	26	Parastacidae	69
Cyprididae	23	Perthidae	78
Cypridopsidae	5	Pezidae	76
Daphniidae	1	Physidae	48
Darwinulidae	65	Pionidae	30
Dytiscidae	50	Planorbidae	66
Ecnomidae	51	Pleidae	67
Ephydridae	59	Pomatiopsidae	52
Eylaidae	53	Ptilodactylidae	56
Glossiphoniidae	29	Pyralidae	66
Halicaridae	64	Sididae	52
Haliplidae	62	Simuliidae	62
(Harpacticoida)	61	Sphaeriidae	53
Helodidae	66	Stratiomyidae	48
Hydrachnidae	22	Succineidae	57
Hydrobiidae	76	Tabanidae	58
Hydrodromidae	66	Tanypodinae	71
Hydrophilidae	60	Thaumauleidae	66
Hydroptilidae	67	Tipulidae	35
(Hydrozoa)	64	(Turbellaria)	63
Leptoceridae	50	Unionicolidae	58
Lestidae	63	Veliidae	60

the other values. Although total scores close to the maximum possible (700-800) should indicate high ecological health, thresholds of classification were not provided.

Table 12 - Indicative interpretation of WBI scores for wetlands on the Swan Coastal Plane.

WBI	Interpretation for Swan Coastal Plain wetlands, Western Australia
< 50	Severe eutrophication or the impact of other human activities is likely.
50 – 60	Eutrophication or the impact of other human activities may be present (more investigation is needed).
> 60	Eutrophication is unlikely (but the possible presence of an unusual human impact to which SWAMPS is not sensitive should not be ignored).

The authors admitted that this index should be intended as preliminary until its validation is accomplished by incorporating data from a wider range of lagoon environments.
However there are two possible criticisms: firstly it is unlikely to find a reference site within each ecosystem considered that provide the best values for the metrics, therefore each studies should be required a homologous reference site; secondly it is questionable to attribute positive contribution to high biomass of phytoplankton and seaweed, especially in presence of blooms due to eutrophication.

4.8 AZTI’ Marine Biotic Index (AMBI)
The AZTI’ Marine Biotic Index (AMBI, also known as Biotic Coefficient, BC) was developed by Borja *et al.* (2000) for European coastal waters. AMBI is based on the classification of species in five ecological groups, as previously proposed by some other authors (Glémarec and Hily, 1981; Glémarec, 1986; Majeed, 1987; Grall and Glémarec, 1997), and their distribution along an organic pollution gradient, according to the ecological succession in stressed environments (Pearson and Rosenberg, 1978; Fig. 6). The ecological groups (EGs) was defined as:

• EG_I: species very sensitive to organic enrichment and present under unpolluted conditions (initial state). They include the

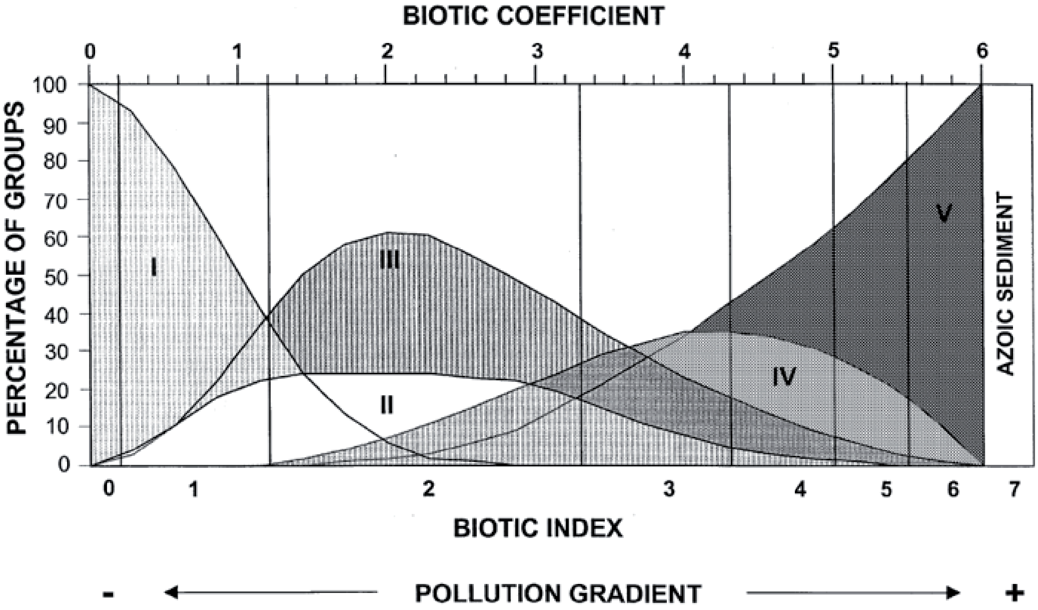

Figure 6. Theoretical model, which provides the ordination of soft-bottom macrofauna species into five ecological groups, according to their sensitivity to an increasing pollution gradient (Borja et al., 2000).

specialist carnivores and some deposit-feeding and tube-dwelling polychaetes.

• EG_{II}: species indifferent to enrichment, always present in low densities with non-significant variations with time (from initial state, to slight unbalance). These include suspension feeders, less selective carnivores and scavengers.

• EG_{III}: species tolerant to excess organic matter enrichment. These species may occur under normal conditions, but their populations are stimulated by organic enrichment (slight unbalance situations). They are surface deposit-feeding species, as tube-dwelling spionids.

• EG_{IV}: 2nd order opportunistic species (slight to pronounced unbalanced situations).
Mainly small sized polychaetes: subsurface deposit-feeders, such as cirratulids.

•EG_{V}: 1st order opportunistic species (pronounced unbalanced situations). These are deposit-feeders, which proliferate in reduced sediments.

Benthic invertebrates are classified into ecological groups according to a checklist regularly updated by some scientists. In October 2005 the list included 3459 taxa.
The index is calculated by the formula:

$$AMBI = \frac{0 \times \%EG_I + 1.5 \times \%EG_{II} + 3 \times \%EG_{III} + 4.5 \times \%EG_{IV} + 6 \times \%EG_V}{100}$$

The index can assume value in the range 0-6, while the value 7 is attributed to azoic samples. The AMBI represent the benthic community "health" and its interpretation as "pollution or disturbance" classification of a particular site and ecological status (sensu European Water Framework Directive) is given in Table 13.
Last updated list of taxa and calculation software, with some graphical function, are freely available on Internet at the address: http://www.azti.es/.
Actually, the idea to obtain a biotic index from the relative abundances of benthic

Table 13 - Summary of the AMBI values and their equivalences (from Muxika et *al.*, 2005).

Biotic coefficient	Dominating ecological group	Benthic community health	Site disturbance classification	Ecological status
$0.0 < AMBI \leq 0.2$	I	Normal	Undisturbed	High status
$0.2 < AMBI \leq 1.2$		Impoverished		
$1.2 < AMBI \leq 3.3$	III	Unbalanced	Slightly disturbed	Good status
$3.3 < AMBI \leq 4.3$		Transitional to pollution	Moderately disturbed	Moderate status
$4.3 < AMBI \leq 5.0$	IV-V	Polluted		Poor status
$5.0 < AMBI \leq 5.5$		Transitional to heavy pollution	Heavily disturbed	
$5.5 < AMBI \leq 6.0$	V	Heavy polluted		Bad status
$6.0 < AMBI \leq 7.0$	Azoic	Azoic	Extremely disturbed	

invertebrate divided in sensitive groups was previously developed by Word in the late '70 years (Ferraro *et al.*, 1991 and references therein). He developed an Infaunal Index for the Southern California coast based on the relative proportion of 53 invertebrate divided in four groups (I: pollution-sensitive; II: slightly pollution-tolerant; III: moderately pollution-tolerant; IV: pollution-tolerant). The AMBI index was applied to several ecosystems, from coastal continental shelf to estuaries and coastal lagoons, along European coast (Atlantic Ocean, Baltic Sea, Mediterranean Sea, North Sea, and Norwegian Sea), even in Hong Kong, Uruguay and Brazil, and toward different impact source, including drill cutting discharges, submarine outfalls, harbour and dyke construction, heavy metal inputs, eutrophication, engineering works, diffuse pollutant inputs, recovery in polluted systems under the impact of sewerage schemes, dredging processes, mud disposal, sand extraction, oil spills, fish farming (Borja *et al.*, 2000; Borja *et al.*, 2003; Borja *et al.*, 2003; Muxika *et al.*, 2003; Ponti *et al.*, 2003; Gorostiaga *et al.*, 2004; Ponti and Abbiati, 2004; Salas *et al.*, 2004; Muniz *et al.*, 2005; Muxika *et al.*, 2005 and references therein). A long debate on the application of this index in the context of the European Water Framework Directive was published on the scientific journals (Borja *et al.*, 2004; Borja *et al.*, 2004; Borja *et al.*, 2004; Simboura, 2004; Dauvin, 2005). Main advantages of this index are that it is simple to calculate and interpret, it does not require any local and/or simultaneous reference site, and moreover it provides a series of continuous values suitable for statistical analyses. The main critic to this index is that the sensibilities of the taxa are based on expertise judgements rather than experimental evidence. Furthermore the sensitivity and recoverability of a species toward different physical chemical and biological factors (e.g. substratum loss, change in hydrodynamics and sedimentation, turbidity, temperature, salinity, oxygenation, desiccation, heavy metal, hydrocarbon, radionuclide, nutrient, introduction of microbial pathogens/parasites, introduction of non-native species, removal of species, etc.) can not be generalised in an univocal group (Tyler-Walters *et al.*, 2001; Ponti *et al.*, 2003; Ponti and Abbiati, 2004). In practice when the assemblages include several species, which are assigned to the ecological groups, misclassification could be compensated each other and therefore the index appeared more robust. As suggested by the authors, when the percentage of taxa that are not assigned is high (>20%), the results should be evaluated with care but if it exceed 50%, the AMBI should not be used (Borja and Muxika, 2005).

Although azoic situation is theoretically considered, the robustness of the index could be reduced when only a very low number of taxa (1–3) and/or individuals (<3 per replicate) are found in a sample (Borja and Muxika, 2005). Unfortunately, situations with very low number of taxa and/or individuals are fairly common in some particular impacts (e.g. sand extraction, dredged sediment dumping, fish trawling, etc.) and even in some naturally-stressed locations (e.g. naturally organic matter enriched bottoms, Zostera beds producing dead leaves, inner parts of estuaries with low-salinity, etc.).

In some cases naturally organic matter enriched bottoms can lead to a natural increase in opportunistic species and, subsequently, to an increase in the AMBI values, providing "wrong" classifications. In order to minimise misclassification problems and obtains a more comprehensive view of the benthic community, the authors recommend the use the AMBI together with other metrics, such as diversity and richness, following a multimetric approach, especially for the purpose of "Ecological Status" definition under the European Water Framework Directive.

Guidelines suggested by the authors (Borja and Muxika, 2005) are:

• AMBI index is designed only for use with soft-bottom communities, never use it with hard-bottom substrata data and remove from the dataset all non-soft sediment taxa (e.g. Nudibranchia) or epifaunal taxa (e.g. Bryozoa).
• remove from the dataset all non-benthic invertebrate taxa (e.g. fish, algae, and planktonic taxa);
• remove all freshwater taxa (e.g. Cladocera);
• in salinity >10 psu, remove insecta;
• remove juveniles, when the species are not identified;
• certain taxa should be grouped together (e.g. species of the same genus not well recognised);
• never use high taxonomic levels (e.g. Bivalvia, Gastropoda), except those included in the taxa list (e.g. Nemertea, etc.);
• use le latest version of the taxa list;
• it is preferable to calculate the AMBI values for each of the replicates, then to derive the mean value;
• never apply AMBI automatically, more detailed analysis and discussion of the results by the experts involved in the assessment is recommended.

4.9 The BENTIX biotic index

BENTIX index was based on the same methodological approach of AMBI (Simboura and Zenetos, 2002).

The ecological groups involved in the formula were reduced from five to three. In the opinion of the authors this reduction should avoid errors in the grouping of the species, and reduce effort in calculating the index, without at the same time loosing its discriminative power or sensitivity. The ecological groups were described as:

• EG_I: species sensitive to disturbance in general. These species corresponds to the K-strategy species, with relatively long life, slow growth and high biomass (Gray, 1979). Also species indifferent to disturbance, always present in low densities with non-significant variations with time are included in this group, as they cannot be considered as tolerant by any degree.

• EG_{II}: species tolerant to disturbance or stress whose populations may respond to enrichment or other source of pollution by an increase of densities (slight unbalanced situations). Also this group includes second-order opportunistic species, or late successional colonisers with r-strategy: species with short life span, fast growth, early sexual maturation and larvae throughout the year.

• EG_{III}: this group includes the first order opportunistic species (pronounced unbalanced situations), pioneers, colonisers,

or species tolerant to hypoxia.
Benthic invertebrates are classified into ecological groups according to a checklist of 350 taxa provided by the authors (Simboura and Zenetos, 2002).
The index is calculated by the formula:

$$BENTIX = \frac{6 \times \%EG_{I} + 2 \times \left(\%EG_{II} + \%EG_{III}\right)}{100}$$

The BENTIX can produce a series of continuous values from 2 to 6, being 0 when the sediment is azoic. Numeric values between 2 and zero are nonexistent in the scale because if EG_I is zero the BENTIX index is 2. A classification system of soft bottom macrozoobenthic communities was proposed based on the BENTIX index and including five levels of ecological quality, as required by the European Water Framework Directive (Table 14).
BENTIX was already applied to some Greek study case in order to meet the EU Water Framework Directive (Simboura *et al.*, 2005). Similarly to AMBI, BENTIX effectiveness could be strongly affected by the degree of confidence in the attribution of generic sensitivities groups, which does not consider the typology of disturbance. Moreover, the formula, in practice, joins EG_{II} and EG_{III} in a unique group. That prevents to distinguish between the relative abundances of these two groups and probably reduced the sensibility of the index.

Tabel 14 - Classification scheme of soft bottom benthic habitats.

Pollution Classification	**BENTIX**	**Ecological Quality Status**
Normal/Pristine	4.5 - 6.0	High
Slightly polluted	3.5 - 4.5	Good
Moderately polluted	2.5 - 3.5	Moderate
Heavily polluted	2.0 - 2.5	Poor
Azoic	0	Bad

4.10 Benthic Response Index (BRI) for southern California continental shelf
Although the Benthic Response Index (BRI) for southern California mainland shelf was not developed for transitional waters deserve attention. It is based on species sensitive/ tolerance, which was determined based upon their distribution of abundance along the pollution gradient between impaired and reference sites (Bergen *et al.*, 2000; Smith *et al.*, 2001). Reference condition was established as the index value in samples taken distant from areas of anthropogenic activity and for which no contaminants exceeded the effects range low (ERL; Long *et al.*, 1995) screening levels. Four response levels were established as the index values at which key community attributes were lost. Pollution tolerance scores to each species were assigned in an objective way, after a multivariate analysis and on the position take along the gradient defined in the ordination space.
The index formula for each sample is:

$$BRI = \frac{\sum_{i=1}^{n} p_i \sqrt[3]{a_i}}{\sum_{i=1}^{n} \sqrt[3]{a_i}}$$

where n is the number of species for sample, p_i is the pollution tolerance score for species i, and a_i is the abundance of ith species. Species in the sample without pi values are ignored. The authors provide a list of 519 taxa with scores differentiated for shallow, middle and deep waters (http://www.esapubs.org/archive/appl/A011/014/appendix-B.htm).

4.11 Multimetric Benthic Indices of Biotic Integrity (B-IBI family)
The benthic indices of biotic integrity could be considered as a family of multimetric indices that evaluates the ecological health by comparing values of key benthic community attributes to reference values expected under

non-degraded conditions in similar habitat types. It is therefore a measure of deviation from reference conditions. This approach was first introduced for freshwater fish assemblages and is frequently referred as Index of Biotic Integrity (IBI; Karr, 1981; Karr *et al.*, 1986).
The progenitors developed for coastal benthic assemblages were the Benthic Index of Environmental Condition of Gulf of Mexico Estuaries (Engle *et al.*, 1994) and the Chesapeake Bay Benthic Index of Biotic Integrity (generally known as B-IBI; Weisberg *et al.*, 1997).
Afterward several variants were proposed for other northeastern American regions (Fig. 7). Most of these indices were developed within the US EPA's Environmental Monitoring and Assessment Program (EMAP) in order to establish water quality criteria and standards under the American Clean Water Act (CWA), to protect aquatic life from the effects of pollution (Gibson et al., 2000).

4.11.1 Benthic Index of Environmental Condition of Gulf of Mexico Estuaries

The Benthic Index of Environmental Condition of Gulf of Mexico Estuaries in its first version was a linear combination of three metrics: Shannon's diversity index, the proportion of total benthic abundance as tubificid oligochaetes and the proportion of total benthic abundance as bivalve molluscs (Engle *et al.*, 1994).
Afterwards a refinement was proposed, including the abundances of capitellid polychaetes and amphipods as additional metrics; Engle and Summers, 1999; Engle, 2000).
The final formula, obtained after a selection of several metrics using the discriminant analysis, is:

$$DS = 1.5710 \cdot ED - 1.0335 \cdot T - 0.5607 \cdot C - 0.4470 \cdot B + 0.5023 \cdot A$$

where ED is the proportion of expected diversity, T is the mean abundance of tubificid oligochaetes, C the percent of capitellid polychaetes, B the percent of bivalves, and A the percent of amphipods. The ED is based on the expected Shannon's diversity index (log 2 based) calculated in function of the salinity. The calculation procedure, based on three replicate samples, requires transforming and standardising all the metrics before the DS calculation. Finally the Engle's B-IBI was obtained normalising between 0 and 10 the DS considering the minimum and the range observed in the original test data used to develop the index.
Details on the calculation method were provided in Engle (2000). Values less of 3 represented degraded sites while almost pristine sites had values greater then 5. It was applied for several year to Louisianian and Florida estuaries, also comparing the results to other biological measures, like fish tissue contaminants (Macauley *et al.*, 1999; Macauley *et al.*, 2002).

Figure 7 - Biogeographic province where the indices belonging to the B-IBI family were developed, within the US EPA's Environmental Monitoring and Assessment Program (EMAP).

4.11.2 Chesapeake Bay Benthic Index of Biotic Integrity

The Chesapeake Bay Benthic Index of Biotic Integrity includes 4 to 7 metrics selected separately for each of seven habitat typologies, defined on their salinity and sediment mud content (tidal freshwater, oligohaline, low mesohaline, high mesohaline sand, high mesohaline mud, polyhaline sand and polyhaline mud; Weisberg *et al.*, 1997). Metrics used represents measures of species diversity, productivity, species composition, depth distribution, and trophic composition. Shannon's diversity index, abundance, biomass and abundance of "pollution-indicative" taxa were considered for each habitat, while abundance of "pollution-sensitive" taxa was included in all habitat typologies except for tidal freshwaters.

A list of pollution indicative and sensitive taxa were provided (Weisberg *et al.*, 1997). For each metric a score of 5, 3, or 1 is assigned according the corresponding thresholds (see table 7 in Weisberg *et al.*, 1997).

The B-IBI is obtained by computing the mean score across all metrics. Assemblages with an average score less than 3 are considered stressed.

The B-IBI applied in the Chesapeake Bay appeared sensitive, stable, robust, and statistically sound (Alden *et al.*, 2002). Afters years of application in monitoring programs, a slightly different classification range was proposed (Llanso *et al.*, 2003).

4.11.3 Carolinian Benthic Index of Biotic Integrity

The Carolinian Benthic Index of Biotic Integrity was derived for the south-eastern USA estuaries in the same way of the original Chesapeake Bay B-IBI (Van Dolah *et al.*, 1999). This variant considered only four metrics, the same for each habitat typology (mean number of taxa for 0.04 m^2, mean abundance for 0.04 m^2, dominance as 100 minus percent abundance of two most dominant taxa and percent sensitive taxa grouped at genera or family level), but with habitat-specific thresholds.

4.11.4 Benthic Index of Estuarine Condition for the Virginian Biogeographic Province

The Benthic Index of Estuarine Condition for the Virginian Biogeographic Province consisted of three metrics selected among forty-eight candidate metrics by discriminant analysis (Paul *et al.*, 2001): salinity-normalized Gleason's D diversity index (SNGD), abundances of spionid polychaetes (SA) and salinity-normalized tubificid oligochaetes (SNTA). The index is calculated through the formula:

$$BI = 0.0489 \cdot SNGD - 0.00545 \cdot SNTA - 0.00826 \cdot SA - 2.20$$

where

$$SNGD = \frac{100 \cdot GD}{(4.283 - 0.498 \cdot SB + 0.0542 \cdot SB^2 - 0.00103 \cdot SB^3)}$$

GD is Gleason's D diversity index (D= S/lnN) based on infauna and epifauna, SB is the bottom salinity, TA the tubificid abundance, SA the spionid abundance, and (for more details see Paul *et al.*, 2001). Reference sites shown index values >0 while degraded sites had values ≤ 0. Applying this index, the Salinity, as habitat typology descriptor, is directly included in the formula. An adjusted formula for cases where there fewer than three replicate grabs was also provided.

4.11.5 Eaton's biocriteria for North Carolina Estuarine Waters

The Eaton's (or Farrell's) Biocriteria for North Carolina Estuarine Waters (Eaton, 2001) is a multimetric index that combine the total taxa richness (TT), the amphipod and caridean shrimp taxa richness (A&C) and a biotic index based on species sensitivities

(EBI). While the previous described B-IBI indices are based on a quantitative box-corer or grabs sampler, this method required a D frame dip net with a 600-700 µm mesh bag, and a continuously sweep for 10 min. The fauna are semi-quantitatively sorted in five classes of abundance. The EBI is calculated from the individual taxa sensitivity values (according to the list provided by the authors and ranging from 1 to 5) with the formula:

$$EBI = \frac{\sum SV_i N_i}{N}$$

where SV_i is the sensitivity value of the ith taxa, N_i the semi-quantitative abundance of the ith taxa (recorded as 1, 3, 10, 30 or 100), and N is the semi-quantitative sum of individuals in the sample. The classification of the impacts are attributed summing the score [1-5] assigned to TT, A&C and EBI according the thresholds separately provided for polyhaline (S ≥ 35 psu) and mesohaline waters (8 to 20 psu), and two bonus points for some habitat characteristics.

4.11.6 Multimetric benthic assessment in San Francisco Estuary

The multimetric assessment methods proposed for San Francisco Estuary considered total number of taxa, total abundances, oligochaete abundances, number of molluscan taxa, number of amphipod taxa, and Capitella capitata and Streblospio benedicti abundances (Thompson and Lowe, 2004). Assemblages were divided according to two habitat typologies based on salinity range: polyhaline and mesohaline. The “assessment value” (AV) of benthic health is obtained as number of metrics that exceeded the reference range values (Table 15). Samples with two indicators outside their reference ranges (AV = 2) were considered to be slightly impacted, samples with AV = 3 were considered to be moderately impacted, and samples with AV = 4 or 5 were considered to

Table 15 - Reference ranges for benthic assessment indicators in two benthic assemblages in the San Francisco Estuary, divided for polyhaline and mesohaline waters (Thompson and Lowe, 2004).

	Polyhaline		Mesohaline	
	Min	Max	Min	Max
No. taxa	21	66	6	18
Total abundance	97	2'931	20	1'090
No. amphipod taxa	2	11		
Molluscan taxa			1	4
Oligochaete abundance			0	47
Capitella capitata	0	13		
Streblospio benedicti			0	38

be severely impacted. Selection of candidate metrics was based on the literature concerning similar habitat typologies, while the definition of reference range values (min and max) were obtained from local “reference” samples. There are not locations free of sediment contamination in the estuary, sediment toxicity is widespread and persistent, and moreover no other estuaries along the central California coast are similar to the San Francisco Estuary enough to be suitable as reference locations. Therefore a screening procedure was used to identify benthic samples that showed no evidence of benthic impacts based on co-occurring sediment toxicity data and on expected species composition at reference benthic conditions as reported in the literature from other areas (Thompson and Lowe, 2004). There are some important constrain that limits the applicability of all the B-IBI family indices. Firstly they are explicitly developed for specific biogeographic regions considering local reference conditions. Application of similar approach for derive equivalent B-IBI in other regions required the presence of some local reference sites. Within all variant of the B-IBI, an important role is attributed to “pollution-indicative” and/or “pollution-sensitive” taxa, based on local checklists or to the unlikely general assumption that higher taxonomic groups, like tubific oligochaetes, capitellid or spionid polychaetes, and amphipods or caridean shrimps have homogeneous and consistent response to any anthropogenic disturb.

4.12 Forthcoming indices

Some new benthic biotic indices oriented to transitional waters were recently presented in national and international conferences held in Europe. Two of them are already under field evaluation and promise to be available soon.

4.12.1 Fuzzy INdex of Ecosystem integrity (FINE)

Fuzzy INdex of Ecosystem integrity (FINE) is an end-user oriented multimetric biotic index based on the "fuzzy-logic" and specifically developed for transitional ecosystems (Mistri *et al.*, 2005). FINE include functional and structural descriptor of the ecosystem with particular emphasis to macrobenthic components. Fuzzy logic basically consists in a way to combine the various interpretations of descriptors that overcome the limits of simple linear combination of scores. This approach can easily tolerate imprecision, allows linguistic reasoning and manage qualitative information (Silvert, 2000). In the proposed index there are 2032 classification rules that finally provide an easily readable interpretation of the ecosystem health.

4.12.2 Index of Size Distribution (ISD)

The Index of Size Distribution (ISD) is a non taxonomic biotic index proposed by Reizopoulou and Nicolaidou (Reizopoulou and Nicolaidou. A., 2004; Reizopoulou and Nicolaidou. A., in press). It is based on the concepts that individual sizes are an intrinsic characteristic of the communities and could be expression of selective pressures or external environmental forces. Generally mean sizes decreased in polluted environments.

Macrobenthic assemblages were sorted in geometric size classes (0.1, 0.3, 0.7, 1.5, 3.1, 6.3, 12.7, 25.5, 51.1, 102.3, 204.7, 409.5 mm). ISD is calculated as skewness of the size frequency distribution. A classification of ecological status is obtained from the threshold provided in table 16.

5 Benthic macrophyte

5.1 Ecological Evaluation Index (EEI)

Ecological Evaluation Index (EEI) was proposed by Orfanidis and colleagues (Orfanidis *et al.*, 2001, 2003). It was designed to estimate the ecological status classes (ESC) of marine benthic macrophytes of transitional and coastal waters. It is based on the well-known pattern that chronic anthropogenic stress, e.g. eutrophication, pollution, shifts the ecosystem from pristine where phanerogams is dominant to degraded state, where opportunistic species through rapid growth and recruitment is dominant. This pattern can be explained from the species competition abilities under abundant resource conditions and is in accordance to *r*- and *K*-selection theory.

The EEI evaluates shifts in marine ecosystem by classifying marine benthic macrophytes from their life-cycle strategy in two Ecological State Groups (ESG_I = most *K*-selected species, ESG_{II} = most r-selected species). This classification scheme is based on the functional-form model of Littler & Littler (1980). ESG_I includes seaweed species with a thick or calcareous thallus, low growth rates and long life cycles (late successionals), whereas the ESG_{II} includes sheet-like and filamentous seaweed species with high growth rates and short life cycles

Table 16 - ISD Interpretation.

Ecological status	***ISD***
High	*ISD* < 1
Good	1 < *ISD* < 2
Sufficient	2 < *ISD*< 3
Poor	3 < *ISD* < 4
Very bad	4 < *ISD*

(opportunistic). All seagrasses are included in the first group, whereas Cyanophyceae and species with a coarsely branched thallus are included in the second group. The evaluation of five ESC needs a cross comparison in a matrix of the ESG_s and a numerical scoring system (Fig. 8).

In order to evaluate the spatial scale-dependent ESC of the studied lagoon, the area-weighted value was calculated. For this purpose, the score of each site was multiplied by the percentage of the lagoon area for which is considered to be representative and the products were summed (Table 17). EEI values higher than 6 indicate sustainable ecosystems of good or high ESC, whereas EEI values lower than 6 indicate that the ecosystems should be restored to a higher ESC (Table 17).

For WFD purpose the biological parameters should be expressed as a numerical value between 0 (bad ES) and 1 (high ES) resulting rom the ratio of the observed value versus the value of the same metric under reference conditions (Ecological Quality Ratio- EQR). The principal of EQR for the case of the EEI could be applied following the formula (Panayotidis *et al.*, 2004):

EEI_{EQR} = 1.25x(EEIvalue/RCvalue)-0.25
where RCvalue = 10

The EEI was designed to cover the prerequisites of European WFD and to offer to water managers worldwide a tool for comparing, ranking and setting management priorities at different spatial levels without a demand for specialized knowledge in seaweed or seagrass taxonomy. EEI was successfully used as status index in five Greek lagoons and the comparison with diversity indices, considered inappropriate for ecological assessment, is shown in Fig.9. These lagoons are located in the Eastern Macedonia & Thrace region, where one of the most extensive Greek fresh water-estuarine systems exists. Nestos River lagoons (Vassova, Eratino, Agiasma, Keramoti) typically consist of a shallow (up to 1.5 m) area and several artificially constructed channels (up to 3 m depth). The fresh water sources of the lagoons are

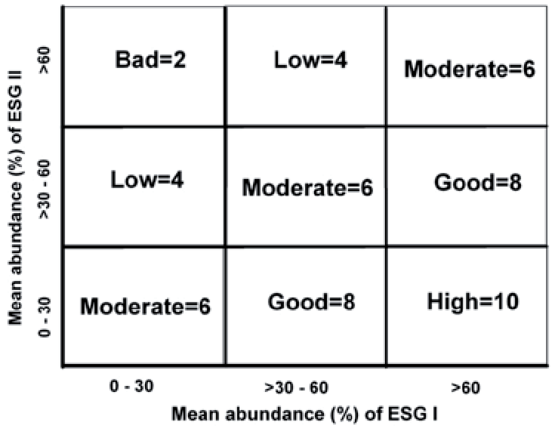

Figure 8. A matrix based on the mean abundance (%) of ESG's to determine the ecological status classes of transitional and coastal waters. Each ecological status class corresponds to a numerical value.

Table 17 - Classification scheme of transitional and coastal waters based on the Ecological Evaluation Index (EEI).

Classification of anthropogenic stress	Ecological status classes	Ecological Evaluation-EEI index range	Management target
Normal/Pristine	High	10 < *EEI* < 8	Sustainable
Slightly stressed, transitional	Good	8 < *EEI* < 6	Sustainable
Moderately stressed	Moderate	6 < *EEI* < 4	Restoration
Heavily stressed	Low	4 < *EEI* < 2	Restoration
Before azoic	Bad	2	Restoration

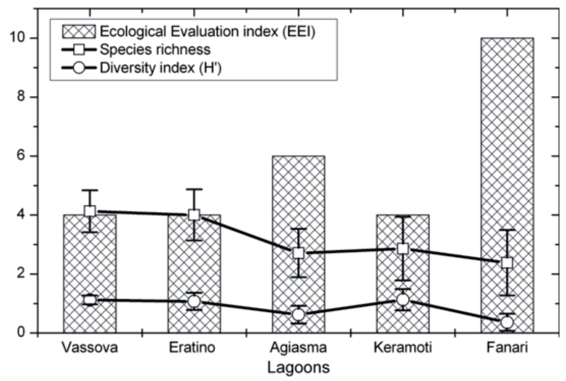

Figure 9. Ecological Evaluation Index (EEI) in comparison to diversity indices in five Greek lagoons of Eastern Macedonia & Thrace regions. High EEI values correspond to the less affected lagoons. Line bars indicate 95% confidence intervals.

mainly agricultural run-offs coming in from surrounding drainage channels and the old bed of the Nestos River. The Fanari lagoon consists of a uniform shallow area (up to 2 m depth) having a narrow connection to the sea. The main fresh water sources of the lagoon are the autumn-winter rainfalls. All lagoons are used for extensive fish cultivation. Since EEI typology is based on ecological processes it can also predict restoration potentialities. According to the model, a restoration goal of a degraded aquatic environment could include an improvement of hydrological and ecological conditions to allow growth of seagrasses in the lagoons, e.g. *Ruppia*, *Zostera*. The value of these communities is high because they support many ecosystem services, e.g. sustain biodiversity, maintain fish habitat, offer detritus to the trophic chain, maintain water quality, stabilize sediment and control erosion.

The index should be calibrated against key abiotic factors of water column and sediment at different spatial scales from lagoon to catchments to biogeographical region.

5.2 Rhodophyceae/Chlorophyceae (R/C) and Chlorophyceae/Phaeophyceae (C/P)

These two indices were proposed by Sfriso and colleagues (Sfriso *et al.*, 2002) to estimate trophic levels of shallow ecosystems like lagoons, inhabited by benthic macrophytes.

The indices are based on Rhodophyceae/Chlorophyceae (R/C) ratio and on Chlorophyceae/Phaeophyceae (C/P) ratio. As the trophic state of the lagoon increases the R/C ratio decreases and the C/P ratio increases.

They are empirical ratios without any obvious ecological base. They have been successfully used to estimate the trophic level in the Venice lagoon. Since taxonomical status of seaweed species in the Mediterranean lagoons seems to be not very well explored additional data are needed to assess applicability of this classification scheme in the Mediterranean coastal lagoons.

5.3 Taxonomic diversity indices

Two indices of taxonomic distinctness have been proposed by Clarke & Warwick (Clarke and Warwick, 1998; Clarke and Warwick, 2001) using presence/absence data:

1. Average taxonomic distinctness (Δ+): average taxonomic relatedness of individuals.
2. Variation in average taxonomic distinctness (Λ+): the evenness of the distribution of taxa across the hierarchical taxonomic tree.

Taxonomic distinctness indices were designed (Warwick and Clarke, 1995) to measure biodiversity by providing the relatedness or organisms within a sample. These indices have been shown to be independent of sample size or sample effort and they have been related to functional diversity and to environmental impact.The theoretical basis is that under anthropogenic stress first disappear the species poor-higher taxa leaving an assemblage comprised of groups of relatively closely related species. This effect tend to decrease the average taxonomic distinctness and to decrease the variability of relatedness of species in the taxonomic tree. Average taxonomic distinctness:

$$\Delta^+ = 2\frac{\sum\sum_{i<j}\omega_{ij}}{S(S-1)}$$

Variation in average taxonomic distinctness:

$$\Lambda^+ = \frac{\sum\sum_{i\neq j}\omega_{ij}^{\,2}}{S(S-1)} - \overline{\omega}^2$$

where

$$\overline{\omega} = \frac{\sum\sum_{i\neq j}\omega_{ij}}{S(S-1)} \equiv \Delta^+$$

The taxonomic distinctness indices from benthic macrophytes assemblages have been tested in two different case studies one from the coastal ecosystem of Bay of Fundy, Canada (Bates *et al.*, 2005), and one from the lagoonal ecosystem of Languedoc-Roussillon region, France (Mouillot *et al.*, 2005a). In both case studies it was indicated a low if any efficiency of the indices to indicate anthropogenic stress in benthic macrophytes assemblages. Bates *et al.* (2005) attributed this result to three basic reasons:

1. the filamentous and leaf-like species of seaweeds, e.g. *Ulva*, *Porphyra*, *Ectocarpus*, that get advantage in anthropogenic stressed environments belong in phylogenetically distant clades. Then samples from these sites, instead of what is expected, give higher index scores than the less impacted sites;
2. anthropogenic stress mainly shifts the dominance from perennial to short-lived species without, except in extreme cases, to completely diminish the former;
3. since diversity of certain red algal orders like Ceramiales and Gigartinales is high,

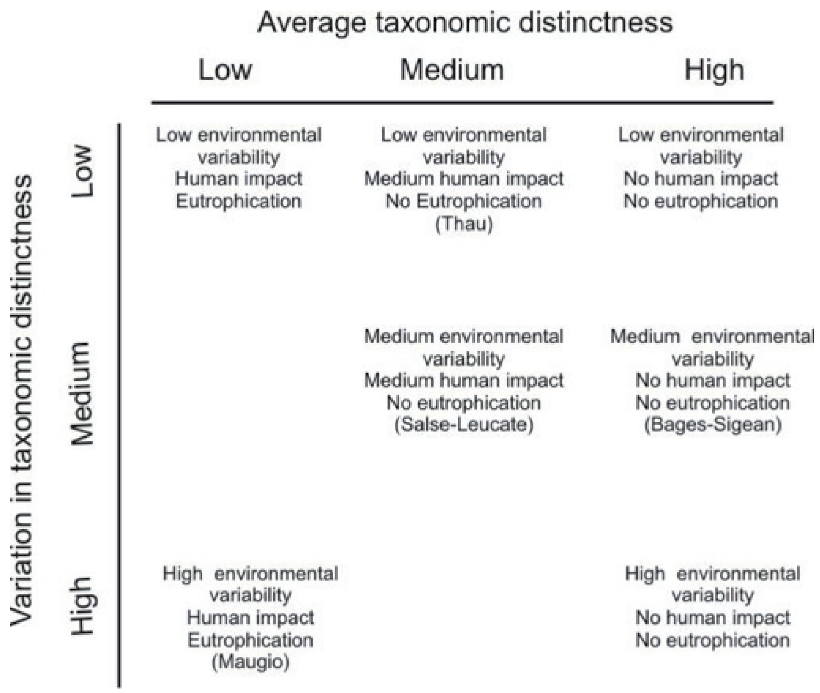

Figure 10. A classification scheme of Mouillot *et al.* (2005a) based on taxonomic diversity indices. Environmental variability is related to the variation of environmental factors such as salinity or temperature. Human impact is mainly related to aquaculture.

there is always a high probability species form that orders to dominate in an assemblage, lowering the indices scores.

A significant highest Δ+ value in one of the less impacted lagoons (Bages-Sigean) and the significant highest Λ+ value (not regarding exotic species) in the most salinity variable lagoon (Salse-Leucate) allowed Mouillot *et al.* (2005a) to suggest a new classification of the lagoons system (Fig. 10). In this system the environmental variability of factors such as salinity was related to variation in taxonomic distinctness and the human activities, and eutrophication levels were related to average taxonomic distinctness. Obviously, additional data are needed to assess applicability of this classification scheme.

5.4 IFREMER's classification scheme

This classification scheme was proposed by Souchu and colleagues from French Research Institute for Exploitation of the Sea (IFREMER; Souchu *et al.*, 2000). It was designed to estimate the eutrophication levels of the French Mediterranean lagoons by using benthic macrophytes as indicators. The macrophyte species were classified in five groups in accordance to their life cycle, functional performance and the associated environmental quality: climax, drifting, opportunistic, exotic and freshwater species. The first group comprises the phanerogams *Ruppia* and *Zostera* and several non-blooming macroalgae, which are considered as representative of non eutrophied high quality conditions. The second group comprises the detached seaweeds, e.g. *Gracilaria*, which are transported by waves and currents and are considered as representative of preserved or moderately polluted conditions. The third and forth groups comprise the bloom forming green algae, e.g. *Ulva* and *Cladophora*, and the exotic species, respectively, which are considered as representatives of bad quality conditions. The fifth group comprises the fresh water genus *Potamogeton*, which is considered as representative of fresh water influenced lagoon sites of high water quality. Souchu *et al.* (2000) accepts that in high quality status sites the macrophyte community is dominated by climax species, mainly phanerogams, with a minor existence of opportunistic green seaweeds. In good status sites the climax species are still the dominant and the opportunistic green macroalgae can only locally proliferate. The moderate status sites are characterized by obvious episodes of macroalgal blooms, anoxic crises and biodiversity loss. In poor quality sites the macroalgal blooms are persistent in cost of seagrasses where disappear, whereas in bad quality sites beside the persistent macroalgal blooms there is strong degradation and frequent dystrophic crises.

The concepts of IFREMER's and Ecological Evaluation Index (EEI; Orfanidis *et al.*, 2001, 2003) classification schemes are very similar accepting the dominance of phanerogams as indicator of high water quality conditions and the dominance of opportunistic, mainly green algae, as indicators of degraded conditions. However, there are also discrepancies regarding the criteria used to classify benthic macrophytes in different ecological groups. Whereas the ecological groups of EEI (ESG I, II) are in accordance to *r*- and *K*- selection theory and to functional-form model of Littler & Littler (1980), the ecological groups in IFREMER's scheme built in a more empirical basis without to follow any obvious ecological theory-model. For example, the group of climax species comprises the phanerogams together with genera like *Bryopsis*, *Scytosiphon*, *Dictyota*, *Ceramium*, *Dasya* etc. that follows very different life-cycle strategies and having very different functional roles in the ecosystem. Drifting seaweeds in the lagoons can be also taxa being typical of climax, like *Cystoseira*, species. However, in the IFREMER's scheme *Ulva* and *Gracilaria* were classified in different groups, whereas in EEI, even provisionally, these two species were grouped together. Although both *Ulva* and *Gracilaria* are common bloom-forming species in lagoon ecosystems the species of *Ulva* rather indicates worst eutrophicated conditions. Obviously additional knowledge is needed in ecosystemic as well as in ecophysiological levels to further improves and specifies both classification approaches. In IFREMER's scheme a numerically improving of assessment approach is also needed.

5.5 Specific macrophyte exergy index

This holistic thermodynamic indicator was proposed by Jørgensen and colleagues (Jørgensen *et al.*, 2005a) where as exergy is defined "the amount of work a system can perform when it is brought into equilibrium with its environment". Exergy is the energy that can be utilized for doing work opposite the heat released at the temperature of the environment that cannot be utilized to do work (Jørgensen, 2002). Specific exergy (Exsp) is the exergy/biomass and is generally considered as a measure of the information contains an organism:

Specific exergy $$\frac{Ex_{sp}}{RT} = \sum \beta_i \frac{c_i}{c_t}$$

where β is the genetic information, ct is the total biomass concentration and ci is the biomass of component i including inorganic matter available for biomass to grow.

In healthy ecosystems the Exsp is high due to dominance of *K*-selected long-lived life forms with generally high information content per unit biomass, i.e. high β-values. By contrast in degraded ecosystems the Exsp is low due to dominance of *r*-selected ephemeral or opportunistic species with generally low β-values.

Exergy and Exsp have been successfully applied as ecological indicators in fresh (Xu *et al.*, 1999; Silow and In-Hye, 2004), transitional (Marques *et al.*, 1997, Marques *et al.*, 1998, Marques *et al.*, 2003; Jørgensen, 2002; Salas *et al.*, 2005) and coastal ecosystems (Jørgensen, 2002).

Macrophyte Exsp was recently used to assess ecosystem health of several Mediterranean French lagoons (Austoni *et al.*, 2006). By using biomass data they successfully estimated Exsp of different ecological status classes in accordance to IFREMER classification scheme (Souchu *et al.*, 2000). The poor and bad ecological status classes were not statistically different with β-values of 96±16 and 113±45, respectively. The β-values of moderate ecological status class (195±58) were approximately twice as high as the bad and poor ecological status classes and significant lower than the good (347±2) and high (534±100) classes.

An estimation of macrophyte β-values of common taxa in EEI, IFREMER's classification schemes succeeded to statistically confirm the ecological grouping classification, however with several discrepancies (Austoni *et al.*, 2006). Although *K*-selected species seems to have bigger genomes than *r*-selected species and therefore higher β-values, the highest reported β-values regard the multinucleate Siphonocladales, like *Valonia utricularis* (1874), V. aegagropila (592), with some of them being characteristic opportunistic taxa, like *Cladophora prolifera* (790), *C. pellucida* (330), *Codium fragile* (2334). Late successional taxa like *Ruppia cirrhosa* (356), *Zostera noltii* (520) and *Z. marina* (422) showed lower or similar β-values.

Very close β-values of *Gracilaria* (132) and *Ulva* (100) taxa seem to justify the classification of both genera in the same ecological group in EEI scheme. Since macrophyte β-values are species-specific any advantage to doubtful taxonomical status of seaweed species in the Mediterranean lagoons regarding sibling and cryptic taxa, as well as geographical ecotypes, could improve the success of Exsp holistic approach to assess a macrophyte-based ecological quality status.

6 Phytoplankton

6.1 Diversity indices

6.1.1 Species Richness and abundance

Species richness and abundance are generally used for describing phytoplankton guild structure, as is often done for the benthic assemblages (refer to 4.1). The most common ecological indices used are:

1. Species richness (S). The number of phytoplankton species observed;
2. Total number of individuals (N);
3. Margalef's species richness (Legendre and Legendre, 1983). Given by the formula:

$$D_{Mg} = \frac{S-1}{\ln N}$$

4. Menhinick's index

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

5. Odum's index for 1000 individuals

$$D_{od} = \frac{SN}{1000}$$

These indices are affected by sample size. The most common index is the number of species. However, it is a non-exhaustive measure of the number of species, since it depends on the level of precision selected for the sample analyses and the concentration of phytoplankton cells in the sample.

6.1.2 Heterogeneity

The term heterogeneity was introduced for the first time by Good (1953). For many ecologists the term is synonymous with diversity. However, it combines species richness and contribution of species populations within the community (evenness).There are many heterogeneity indices but the most frequently used in studies of phytoplankton ecology are Simpson's index (D; Simpson, 1949)) and Shannon's index (H'; Shannon and Weaver, 1949). The formulas for calculating these are as follows:

$$D = \sum \left(p_i^2\right)$$

$$H' = -\sum \left(p_i \cdot \log p_i\right)$$

Shannon's index is mainly used to highlight the importance of rare species, whereas Simpson's index is used to highlight the importance of dominant species. The main advantages regarding the use of these indices, which have ensured their success and their frequency of adoption by the scientific community, are:

1. their ease of calculation
2. the fact that they eliminate the problems related to sample size and comparison of samples from different ecosystems in that they are estimated on the basis of the relative contribution of each taxa in the sample.

Disadvantages include:

1. The range of values that they present is generally very narrow, making it difficult to discriminate if the differences are not pronounced.
2. The taxa included in the estimate must all be classified at the same taxonomic level.

6.1.3 Evenness

Evenness indicates the distribution of the individuals within each species. Evenness can be obtained from the Shannon's index by dividing the value of the index by the maximum diversity value possible for that particular community. This value is calculated as ln S, where S is the number of species observed in the sample (refer to 4.1.3).

Table 18 - Eutrophication scale for each of the seven ecological indices.

Diversity Index	Oligotrophic conditions	Lower limit of mesotrophic conditions	Upper limit of mesotrophic conditions	Eutrophic conditions
S		12	18	24
N		4160	31400	188334
D_{Mg}		1.32	1.50	1.89
D_{Mn}		0.19	0.09	0.05
D_{Od}		3.05	0.15	0.04
H'		1.91	1.41	
E		0.80	0.68	0.45

6.1.4 Case study based on the use of diversity indices for the evaluation of the trophic state of marine-coastal environments.

There are a number of studies in which attempts have been made to use diversity indices for the evaluation of the trophic state of marine-coastal environments.

The study conducted in the Gulf of Saronicos (Kitsiou and Karydis, 2000) is interesting because it also attempts a classification into trophic categories on the basis of the values calculated for a series of diversity indices.

Specifically, the trophic classes (Table 18) were established in relation to the values calculated for each index from the median value derived from a phytoplankton data set obtained from environments that were classified a priori as eutrophic, mesotrophic or oligotrophic. The values calculated for each index are shown below (Table 18).

In a study carried out in the northern Adriatic (Vadrucci *et al.*, 2003), a comparison of four diversity indices showed significant variations in values along trophic gradients (primary production).

6.2 Functional diversity

Functional diversity is unrelated to the taxonomic characteristics of the species; it takes account exclusively of their functional and structural characteristics. These characteristics determine the presence or otherwise of a species within a given ecosystem and consequently determine the structure of the community. The functional approach to the study of phytoplankton corporations is based on the fact that the structure of the phytoplankton corporations is determined by specific functional attributes of the species that render them particularly fit to survive in given environmental conditions Fig. 11). This approach was used for the first time by Margalef, who identified three functional categories for the phytoplankton species (I: *r*-selected summer species, II: mixing tolerant species or vernal or autumnal species, III: *K* selected summer species) and analysed their distribution within a contingency matrix whose axes are represented by trophic availability and hydrodynamism. It was taken up again

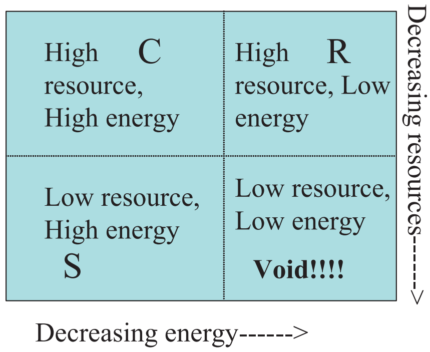

Figure 11 . The Habitat template and the replication strategies.

by Reynolds (1997) who identified three replication strategies in the phytoplankton corporations, indicated as C, R and S (Table 19), which were also allocated in a contingency matrix whose axes were represented by the nutrient availability and light. In this system:

1. C- are the invasive species, which appear in the first phase of the ecological succession with good availability of nutrients and light. They are small and have high replication rates;
2. S- are the le "acquisitive" species; they are of large dimensions or form large colonies. They are typically *K*-selected and grow slowly even under good trophic conditions;
3. R- are species of intermediate dimensions that are stress tolerant, managing to survive well in highly turbulent conditions. They enjoy a competitive advantage in conditions characterised by high availability of nutrients and low availability of light.

These attributes of the species are in the pre-adaptation category and thus tend to be quantifiable features of the species, often strongly correlated with their morphometric characteristics. It is thus possible to identify the functional category of a given species by verifying its bio-volume, its cellular surface area and its maximum linear dimension. The habitat-template is reconstructed on the basis of certain morphometric characteristics (S/V e M/SV). The S/V ratio refers to the ratio between the surface area and the cellular volume; it decreases as cellular volume increases whereas the M/SV ratio refers to the ratio between the maximum linear dimension of a cell and the product of S and V.

This index represents the distortion of the cell with respect to the spherical form, which is also an index of its photo-adaptive potential, i.e. the capacity of the cell to grow in given light conditions (Fig. 12).

However, the use of functional groups as phytoplankton descriptors for transitional aquatic environments is still a long way off. In contrast, many applications can be found in freshwater lacustrine systems, where various phytoplankton assemblages have been identified in association with different chemical and physical characteristics of lakes (Reynolds, 1997, 2003). However,

Table 19 - Characteristics of C, R and S species.

	Morphometric Data		Functional features		Dominance	Typical representative
	V μm^3	S/V μm^{-1}	Replication rate	Efficiency in light adsorption		
C- INVASIVE	$< 10^3$	>0,5	High	Intermediate	After thermal stratification after nutrient input	picoplankton nanoplankton
R- ACCLIMATING	$> 10^3$	>0,5	High	High	deeply circulating water layer	*Asterionella* *Fragilaria* *Tabellaria*
S- AQUISITIVE	$>10^4$	<0,3	Low	Low	Stratified system	*Ceratium* sp. *Microcystis*

this approach has never been applied to transitional aquatic environments. Such an application however could provide interesting information since it may enable a classification of the phytoplankton guilds of transitional aquatic environments, by means of the association of specific taxonomic groupings with particular habitats.

This is also interesting from the point of view of identifying specific communities of references for given types of habitat and understanding how these may be altered in relation to the presence of environmental stresses, including those of an anthropogenic nature. This is also based on the assumption that although many factors, both biotic and abiotic, may influence the structure of a phytoplankton community, even a non-expert reader of phytoplankton is able to determine from what type of environment it was taken, specifically if this environment is

- oligotrophic or eutrophic
- deep or shallow
- primarily freshwater or saltwater
- stratified or mixed

It is also possible to specify with a reasonable degree of accuracy the time of year when the sample was taken.

6.3 The Phytoplankton Index of Biotic Integrity (P-IBI)

This index was developed during the "Chesapeake Bay Water Quality Monitoring" programme (Lacouture *et al.*, 2006).

Dissolved inorganic nitrogen, orthophosphate and Secchi depth were used to characterize the conditions of the habitat. Least-impaired (reference) habitat conditions have low dissolved inorganic nitrogen (DIN) and orthophosphate (PO4) concentrations and large Secchi depths. Impaired (degraded) habitat conditions have high DIN and PO4 concentrations and small Secchi depths. This is an index of the combined type. Thirty different metrics were tested for their ability to discriminate between least-impaired and impaired conditions and twenty of these were classified in order to create community index at four levels of salinity.

The metric scoring approach described by Karr *et al.* (1986), Weisberg *et al.* (1997), Gibson *et al.* (2000), served as a template for the phytoplankton metric scoring. Scoring is based on the distribution of each phytoplankton metric in the reference community. An example of pattern of variations P-IBI is reported in the Fig. 13.

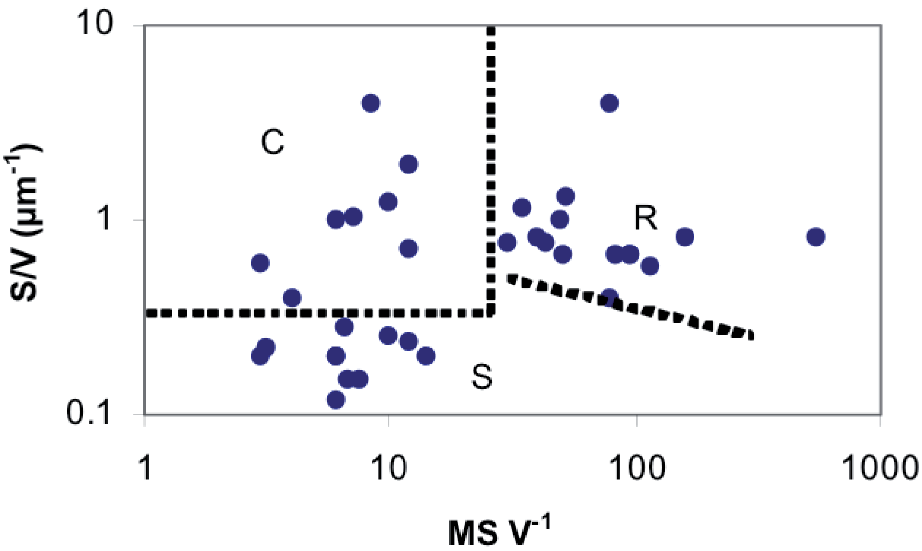

Figure 12 . The Habitat Template in relation to the morphometric characteristics of the cells.

6.4 Biotic indices based on diatoms

The protocols for estimating the quality of the water based on the use of diatoms are now well-developed and their value has been recognised on a national and international level (Sgro and Johansen, 1998). However, they cannot be considered a rapid form of technology because of the time required for the preparation of the samples and the species level analysis. However, as a method it is applicable to all types of aquatic ecosystem, including transitional environments. The details of the assemblages of diatoms provide support for investigations of a paleo-ecological nature, making it possible to perform historic reconstructions of water quality. These approaches however should be understood as specifically referring not to phytoplankton but rather to periphyton, i.e. the micro-algal component that lives attached to the substrates (sediments, stones, etc). The diagnostic attributes attributable to diatoms are the following:

1. They exhibit a broad range of tolerance along productivity gradients and differentiation in the use of trophic resources on the species level;
2. They exhibit a shorter generation time than other indicators (about two weeks). Therefore they reproduce and respond rapidly to environmental changes and are able to provide early measurements both in the impact evaluation phase and in the ecosystem recovery phase.
3. They are sensitive to changes in the concentration of nutrients. Each taxon has a specific tolerance optimum for nutrients such as phosphates and nitrogen, which is also quantifiable.
4. They respond rapidly to eutrophication. Since diatoms are photo-autotrophic organisms, their growth is heavily influenced by the concentration of nutrients and the availability of light.
5. Their speed of migration is very high and the lack of physical dispersion guarantees that there is a brief lag-time between perturbation and response.

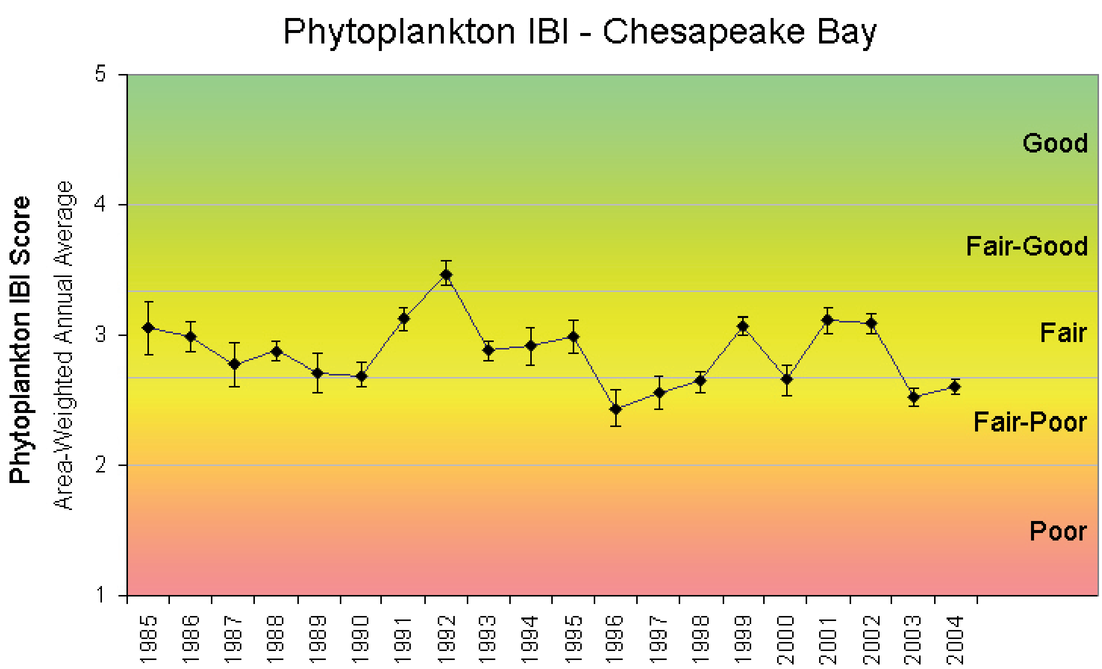

Figure 13 . Pattern of variation of P-IBI in Chesapeake Bay.

6. The diatoms' frustules remain in the sediments for an extremely long time. For this reason, sediment box cores can provide details on the changes in the quality of the waters above. This attribute alone is of great importance, since it concerns not only research into the reference conditions but it may also be important for research into the variations linked to changes in the climate or in the condition of the system before the development of anthropogenic activities.
7. The taxonomy of diatoms is well documented. The identification of the species is based entirely on the morphology of the frustules.

6.4.1 Trophic Diatom Index (TDI)

The TDI is used as an indicator of the trophic state of impacted rivers but it has also been applied to freshwater lakes. This index was developed by comparing levels of reactive phosphorous with the frequency of certain diatom species. The sensitivity value of the species is shown in Table 20.

The index is calculated using the following formula:

$$TDI = \frac{\Sigma a_j \cdot v_j \cdot s_j}{\Sigma a_j \cdot v_j}$$

where: a = the relative abundance of species j in a sample, v = the indicator value for species j, s = sensitivity to pollution of species j.

6.4.2 Lange–Bertalot (LI)

The index classifies the diatoms into three classes:

- Class 1: tolerant to pollution
- Class 2: less tolerant to pollution
- Class 3: sensitive to pollution.

Used in rivers and lakes, but never applied to transitional environments.

6.4.3 Percentage of Sensitive Species Index (SSI)

This metric is calculated from the proportion of sensitive species with respect to the total richness of the sample.

6.4.4 Percentage Tolerant Species Index (TSI)

This metric provides information on the amount of eutrophication that is associated with pollution of the organic type. This characteristic enables the TSI to distinguish between the effects of phosphorous concentration and those of organic pollution (BOD) in an aquatic system. The metric is calculated from the proportion of tolerant species (Table 21) with respect to the total number of species observed.

6.4.5 Generic Diatom Index (GDI)

This index makes it possible to determine the quality of the water directly from the genera. Good results have been obtained from the application of this index in France. The GDI is calculated using the same formula as the TDI:

$$GDI = \frac{\Sigma a_j \cdot v_j \cdot s_j}{\Sigma a_j \cdot v_j}$$

where a = the relative abundance of the genus j in the sample, v = the indicator value and s = the sensitivity to pollution of genus j.

The index ranges from 1 to 5, where 1 corresponds to poor quality status of the water and 5 to the optimal status. The list of genera with the sensitivity and tolerance values is shown in Table 22.

The GDI is one of the most convenient indices used for routine analysis. Both the sampling and the laboratory analyses require only half the time needed to estimate the other indices. Therefore, one of the biggest criticisms of the use of indices based on the taxonomic composition of diatoms for estimating the quality of waters concerns the level of taxonomic knowledge required for the identification at the species level. Although a few months practice should be enough for a technician to acquire this capacity

Table 20 - Species' sensitivity values and ecological status.

Level	*DIP* (mg/l)	Ecological status
1	< 0.01	High status
2	0.01-0.	Good status
3	0.035-0.1	Moderate status
4	0.1-0.3	Poor Status
5	>0.3	Bad status

of identification, the level of ability required for the application of the GDI is much lower.

7 Fish assemblages and mixed indices

7.1 Taxonomic diversity indices of fish

Other than macrobenthic invertebrates, phytoplankton and macrophytes, taxonomic diversity indices can be applied to fish assemblages. However fish species appeared less affected by disturbs than zoobenthic or macrophyte species. Since their mobility they can avoid local and temporary adverse conditions, like extreme temperatures or anoxic crises. Thereafter, they can re-colonize the lagoon as soon as the environmental conditions become more favourable. As a consequence the taxonomic diversity indices of fish communities seem not suitable for assessing the coastal lagoons status or discriminate among different levels of human impact and eutrophication (Mouillot *et al.*, 2005b).

7.2 Estuarine Health Index (EHI)

The Estuarine Health Index (EHI), was developed in South Africa (Cooper *et al.*, 1994). It includes fish assemblages among the biological aspects. It also includes some physical and chemical features related to water quality and geomorphology as well as an additional aesthetic component. It is currently being tested in the UK.

7.3 Estuarine Biotic Integrity (EBI)

An index of Estuarine Biotic Integrity (EBI), based on fish assemblages and also known as "Estuarine Ecological Index", was successfully applied to 16 estuaries on Cape Cod and in Buzzards Bay, Massachusetts, U.S. (Hughes *et al.*, 2002). This EBI is a multimetric index that includes fish abundance, biomass, total species, species dominance, life history, and proportion by life zone. The results carried out in north America indicated that the EBI is sensitive to habitat quality change, although there is a time lag between the degradation and improvement of water quality, fish habitat, and response of the fish community.

7.4 Estuarine Fish Index (EFI)

The Estuarine Fish Index (EFI) is a multimetric biotic integrity index developed for northern European estuaries (Adriaenssens *et al.*, 2002). The methods could be considered an adaptation of the original freshwater fish index of biotic integrity (IBI; Karr, 1981). EFI is based on nine metrics, subdivided in five classes (Table 23), to express the integrated quality of the estuary. Fish are collected using a standard net then analysed for species composition and relative abundance. It has been applied in the upper part of Scheldt estuary where freshwater or low salinity waters are present.

Table 21 - Indicator range.

Proportion of tolerant taxa	Interpretation
<20%	Free from organic pollution
21-40%	Some evidence of organic pollution
41-60%	The organic pollution contributes to eutrophication
>61%	Heavily contaminated by organic pollution

Table 22 - Diatom genera with sensitivity values and indicator value.

Genera	s	v
Achnanthes	5	1
Amphipleura	5	3
Amphora	3	2
Anomoeoneis	5	2
Asterionella	4	2
Caloneis	4	1
Cocconeis	3	1
Cyclotella	3	1
Cymbella	5	1
Denticula	5	3
Diatoma	4	1
Gyrosigma	4	3
Tabellaria	5	1
Thalassiosira	2	3

7.5 Other fish indices

A number of other indices based on fish assemblages were locally developed in some transitional water ecosystem. Unfortunately these attempts are unpublished or described in technical reports difficult to find. Among them the "Fish Health Index" and the "Estuarine Fish Importance Rating" are briefly reported in Jørgensen *et al.*, 2005a.

7.6 Estuarine QUality and condiTION (EQUATION)

The estuarine quality and condition (EQUATION) index is an integrated multimetric indices based on an aggregation of four different components: vulnerability, measuring the physical capacity of the system to react to change, water quality, which examines trophic status and eutrophication aspects, sediment quality, which looks at the sediments and benthic fauna, and trophodynamics, which addresses the quality and value of the top levels of the trophic web (Ferreira, 2000). For each main component there are 5 to 7 metrics to measure or indirectly evaluate and their combination provide a score (Table 24).

The EQUATION index for an estuary is calculated as the weighted sum of the scores obtained for the four main components:

EQUATION = 0.22 vulnerability + 0.26 water quality + 0.26 sediment quality + 0.26 trophodynamics

The index is represented as a number ranging from 5 (better) to 1 (worse), following the European WFD. The index has been tested for a range of estuarine systems, differing in hydrology, tidal characteristics and pollutant load. Five cases have been chosen, two from the USA (San Francisco and Tomales Bay) and three from the northern Europe (Carlingford Lough, Elbe and Tagus estuaries).

A decision support system (DSS) has been developed, integrating the different components. The DSS software runs under Windows NT/98, and is available for download, together with the test systems used, from: http://www.ecowin.org/.

Table 23 -Metrics considered in EFI.

Class	Metric
species composition	total number of species
	key species (selected for each habitat)
trophic composition	opportunists and specialists
habitat use	benthic species
tolerance	tolerance scores
	tolerant species
ecological guilds with estuarine requirements	estuarine resident species
	diadromous species
	marine juvenile migrant species

Table 24 - EQUATION components and descriptors (for more details see Ferreira, 2000).

Component	Objectives	Descriptors
Vulnerability	Quantify system buffering capacity (7 metrics, sampling required for river inflow)	Freshwater residence time (measures flushing of discharges) Estuary number (measures vertical stratification) Tidal prism : volume ratio (measures relevance of tide to the system) Time closed over the year (measures free exchange with ocean)
Water quality	Determine trophic balance based on nutrients, primary productivity and oxygen (6 metrics, sampling required for all)	*DIN* concentration, both conservative and non-conservative (measures eutrophication) Percentage of oxygen saturation (direct measure of water quality)
Benthic quality	Evaluate status of benthos, in terms of biological communities, contamination, and bioaccumulation (5 metrics, sampling required for all)	Sediment contamination (measure of persistent pollutants, such as heavy metals and/or organochlorines) Bioaccumulation (measure of transfer of pollutants to the food chain) Biodiversity (measure of biological condition of the benthos)
Trophodynamics	Assess trophic web equilibrium based on ichthyofaunal data (5 metrics, sampling required for diversity and nursery areas)	Fishing and aquaculture activity (measure of primary sector interest in the system) Quality of fish products (related measure of economic value of the system) Fish diversity (measure of the stability at the top of the trophic web)

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