EP

United Nations
Environment
Programme

UNEP(DEC)/MED WG.231/18
17 April 2003

ENGLISH

MEDITERRANEAN ACTION PLAN

Meeting of the MED POL National Coordinators

Sangemini, Italy, 27 - 30 May 2003

STRATEGIC ACTION PROGRAMME

GUIDELINES

DEVELOPMENT OF ECOLOGICAL STATUS AND STRESS REDUCTION INDICATORS FOR THE MEDITERRANEAN REGION

In cooperation with

GEF

UNEP
Athens, 2003

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1. INTRODUCTION

The importance of indicators for decision making was recognised at the Rio Conference and chapter 40 of Agenda 21 refers to the development of Sustainable Development Indicators (SDI). The indicator activities have often offset in activities in relation to environmental indicators or indicators for sector integration. At the European level the use of indicators is today recognised as one of the most effective techniques for the applied ecological research of the surface and coastal Waters (Ecological Water Quality Directive 2000/60/EC). This Directive is establishing a framework for community action in the field of water policy the so called Water Framework Directive (WFD). The Community and member countries are party to various international agreements containing important obligations on the protection of marine waters from pollution.

The European Environmental Agency (EEA) has established an Inter-Regional Forum (IRF) Working Group to frame the information needs for a potential core set of indicators. Showing ecological status and indicators is a long term goal for ETC/WTR (European Topic Centre on Water), that will be developed over time with the progressive implementation of the WFD by EU Member Countries, taking into account respective differences in terms of level of scales and national monitoring/assessment systems. During an EEA-Marine Conventions joint Workshop on Indicators at JCC (EU Joint Research Centre)-ISPRA, 14-15 June 2001, to optimise the data available by Member Countries and Marine Conventions in producing environmental indicators, it was suggested to Marine Conventions to adopt as much as possible common procedures in their regional indicator assessments, as well as to carry out capacity building activities for harmonisation of data/info collection/management.

Several international organisations have activities to develop frameworks and indicator sets for sustainable development reporting. The developed indicator sets are either aimed for the international organisation own reporting or they are meant as general indicator sets to facilitate national reporting. Environmental indicators are at a very early stage in the Mediterranean, where of a list of 130 indicators (Blue Plan, 2000), only 3, referring to “Economic activities and sustainability”, and those related to fisheries are only developed.

The impacts of human activities on the biological diversity, extending from gene to ecosystem, are most evident in coastal areas. Activities known to affect significantly the biodiversity of coastal ecosystems include shipping (oil spills, exotic species), industry (chemical effluents), dredging and dumping, fishing and mariculture, biological invasions, tourism etc. The effects of eutrophication are excluded here.

This work is based on readily available literature. In addition to review papers, helpful information was extracted from similar projects that are taking place on a national or regional level. In particular, information was gathered from: the Swedish report on Environmental Quality Criteria for coasts and seas (Anonymous 2000); the project Ecosystem Targets North Sea (Bisseling *et al.* 2001) in the Netherlands; the Australian State of the Environment – Environmental indicators report (Ward et al, 1998); the EcoQO’s report for North Sea benthic communities (De Boer et al, 2001) and the BIOMARE concerted action reports. Moreover, the experience was gathered from the EEA work including a series of workshops dedicated to the development of environmental indicators.

This report represents a scientific contribution to the development of EcoQOs for Mediterranean Sea, and lays an important basis for further discussion and progress concerning this topic.

2. AIMS OF THE REPORT

This is an effort to review the state of development of biological indicators and present regional guidelines for the development of ecological status and stress reduction indicators. Specific objectives include:

- presentation of a set of biological/ecological indicators (core set and additional ones) for ecological quality reporting of coastal areas, at both the national level and regional Convention (UNEP-MAP) level in a compatible way;
- sufficient coverage of all major biodiversity issues by the list of proposed (core set) and additional indicators;
- detailed examination of each indicator to ensure that it is rigorously defined, providing also the advantages and limitations in its use;
- identification of relevant data sources for each indicator, if available;
- citation of examples with application of the suggested indicators in defining the ecological status of Mediterranean areas.

3. STATE OF BIODIVERSITY IN THE MEDITERRANEAN

Species Diversity

Biological diversity or biodiversity is a “cluster of concepts” which cover many interrelated aspects from genetics and molecular biology to community structure and habitat heterogeneity. However, the most fundamental meaning of biodiversity probably lies in the concept of species richness (May, 1995), that is the number of species occurring in a site, region or ecosystem.

The Mediterranean fauna and flora have evolved over millions of years into a unique mixture of temperate and subtropical elements, with a large proportion (28 %) of endemic species (Fredj *et al.,* 1992). The present-day variety of climatic and hydrological situations and Mediterranean-specific biotopes, partly due to the geological history of the area, account for the great species variety with few equals in the world (except for the coral reefs). 10 000 to 12 000 marine species have been recorded (with approximately 8 500 species of macroscopic fauna and flora).

Different authors have tried to estimate the total number of marine biota living in the Mediterranean. The “MEDIFAUNE” databank (Fredj et al., 1992) has focused on information on benthic invertebrates but needs updating. The geographical distribution of species is not included in the current compilation of the ERMS (European Register of Marine Species) register [see website reference]. Furthermore, as the ERMS covers all European Seas but not African or Asian waters, it does not have a complete inventory of the species in the Mediterranean and least is known about the eastern basin, a gap identified by DIVERSITAS [Warwick et al., 1996].

The marine species are partly inventoried in France, Italy, Greece and Spain. Progress has been made on a few taxonomic groups at the Mediterranean scale among which Hydrozoa (Boero et al., 1997), Amphipoda (Ruffo, 1998), Sipuncula (Pancucci et al., 1999) and Mollusca (Sabelli et al, 1990-92; CLEMAM website reference).

However, there are still marine major groups such as diatoms whose diversity has been under-estimated because of misbeliefs that “nearly all species are known and global diatom biodiversity is limited” and that “diatom species are cosmopolitan”. Vyverman et al (2001) have demonstrated the potential of diatoms in applied studies and conservation strategies.

Diatoms represent 20-25% of global primary production, are ideal for assessing biodiversity and nature value and are also sensitive proxies for paleoclimate and palaeoenvironmental reconstruction.

Table 1 is a compilation from various sources that has been updated with information from EEA (draft) for marine Invertebrates. This rich biodiversity represents 8 to 9 % of the total number of species in the world's seas. The figures are continuously altered because of new species are still being recorded, especially in hitherto unexplored water layers or geographic areas.

Table 1: No of species within major animal taxa in the Mediterranean (sources: EEA, draft; Bianchi and Morri, 2000; Ruffo, 1998; Boero et al., 1997; Stefanidou, 1996).

Species group	**Species number**	**Species group**	**Species number**
Sponges (Porifera)	622		
Jellyfishes (Hydrozoa)	379		
Sea anemones, corals, rest Cnidaria	100	**Little known groups**	
Sea mat, hornwrack (Bryozoa)	~ 500	Echiurida	6
Segmented worms (Annelida)	1000	Priapulida	3
snails, bivalves, squids and octopuses (Mollusca)	2000	Sipuncula	33
Starfishes and sea urchins (Echinodermata)	154	Brachiopoda	15
Amphipoda	451	Pogonophora	1
Decapoda	340	Phoronida	4
Isopoda	165	Hemichordata	5
Anisopoda	43	Tunicates	244
Cumacea	91		

Ecosystems/Communities

Pelagic

Within the Mediterranean Sea there is a general trend of increasing oligotrophy towards the easternmost part (Levantine sea), expressed as phytoplankton and zooplankton abundance and biomass, as well as of primary production. Recent work has shown that primary production rates are on average three times lower in the eastern than in the north-western basin (Turley, 1999). Primary production rates integrated over the euphotic zone (maximum depth: 120m) were low and about 40, 78 and 155 mg C/m^2 per day in the eastern, central and western Mediterranean basins respectively (Gotsis-Skrettas, unpublished data). Zooplankton abundance in the upper 0-100m layer varied between 93 ind/m3 south of Cyprus and 898 ind/m3 in the Balearic sea in June 1999 (Siokou, unpublished data). On the other hand the Adriatic and the Aegean seas occupy a distinct position within the Mediterranean Sea due to their topography and their hydrology (large fluvial influence in the N.Adriatic Sea, Black Sea water influence in the NE Aegean Sea). These characteristics are reflected in the plankton communities both quantitatively (higher values in the Northern parts- up to 3000 ind/m3 in the 0-50m layer- when compared to the southern parts which communicate with the large Mediterranean basin) and qualitatively (strong neritic component and presence of boreal relict species-the copepod *Pseudocalanus elongatus*- in the northern parts).

About 470 zooplankton species have been recorded in the Mediterranean coastal and offshore waters. Although there is a general idea of decreasing species number (for copepods) from west to east, this could be due to the low sampling effort in the eastern basin. In contrast to the Atlantic, the deep waters of the Mediterranean are characterised by the absence of true deep sea (bathypelagic) species. Instead they are occupied by inhabitants of the intermediate layers (200-500 m) the so-called “mesopelagic” fauna.

Table 2: Summary of the ratios of productivity in the western and eastern basins of the Mediterranean. *Source: Turley, 1999.*

	West/East ratio
Primary production	3.3:1
Bacterial production	1.8:1
Fish production	2.7:1

Benthic

A description of the great variety of ecosystems in the Mediterranean was given by Peres and Picard (1958); it has since been amended by Augier (1982) and Bellan-Santini *et al.* (1994). The basic scheme of classification, based on depth, sediment type, hydrodynamics, light transmission and plant distribution (Figure 1), has been widely adopted by Mediterranean scientists.

The main natural habitat types of community interest in the Mediterranean whose conservation requires the designation of special areas of conservation include: Open sea and tidal areas (7 types-see Table 3); Sea cliffs and shingle or stony beaches (3 types); continental salt marshes and salt meadows (3 types); Salt and gypsum inland steppes (2 types); Sea dunes of the (7 types). Among the most sensitive habitats of the Mediterranean (UNEP RAC/SPA. 1997) we should mention, for the mediolittoral zone, the *Cystoseira* communities: index of hydrodynamism at the upper littoral zone I, the *Posidonia oceanica* meadows in the infralittoral zone, as well as *Lithophylum lichenoides* (sensitive to hydrocarbons) and coralligenous communities (erosion from deliberate tearing off), *Corallium rubrum*: reduction in water transparency due to pollution, turbidity

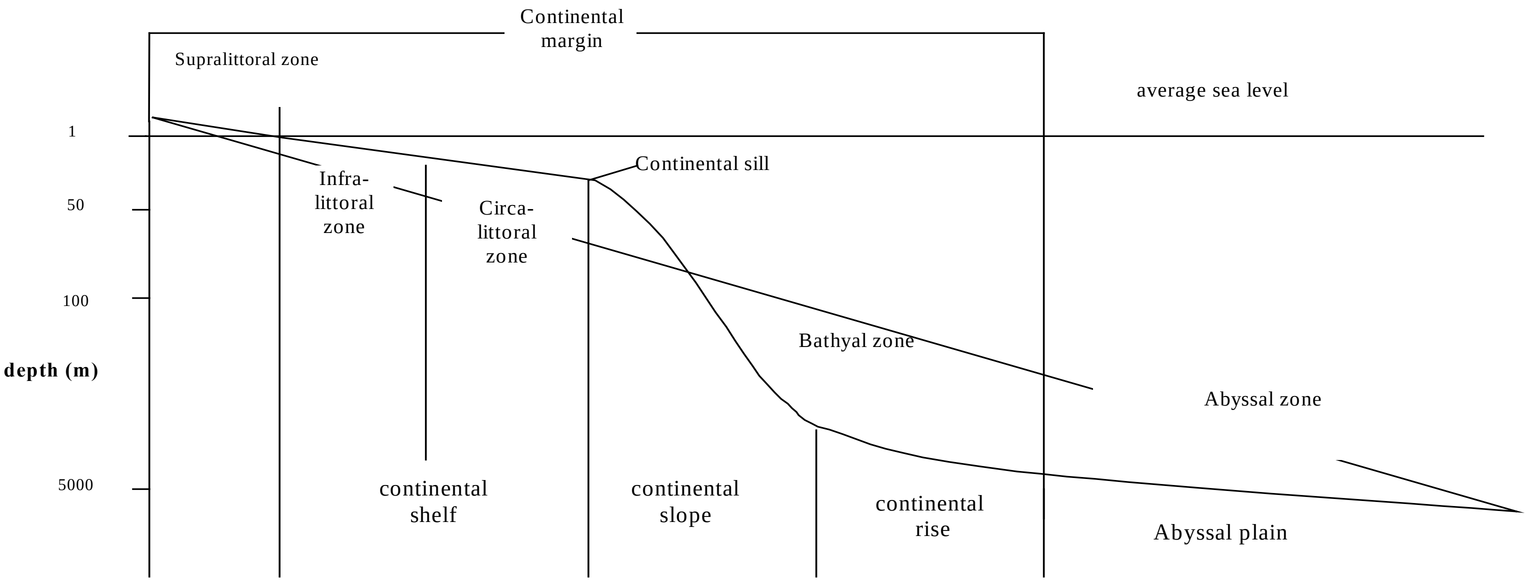

Figure 1: Schematic presentation of the ecological benthic zones as proposed by Perés and Picard, 1964

Table 3: Main marine habitat types in the Mediterranean protected areas under the EU Habitats Directive. *Source:* (http://www.europa.eu.int./comm/environment/nature/hab-en.htm)

- Sandbanks which are slightly covered by sea water all the time
- Posidonia beds
- Estuaries
- Mudflats and sandflats not covered by seawater at low tide
- Coastal lagoons
- Large shallow inlets and bays
- Reefs

Compared with the Atlantic, the Mediterranean marine communities encompass more species with generally smaller individuals (Mediterranean nanism) having a shorter life cycle (Bellan-Santini *et al.*, 1994).

Birds

Wetland loss and habitat degradation are recognised as serious threats for nine out of 33 breeding colonial waterbird species found along the Mediterranean coastline (Erwin, 1996).

Mammals

Among the 22 species of whales (Cetaceans) reported, 10 have only been sighted occasionally and are probably not true inhabitants of the Mediterranean. The other 12 species occur regularly, 8 being common and 4 much less frequent (Beaubrun, 1994). Nineteen of the cetaceans (seals included) are listed in Annex II (List of endangered or threatened marine species in the Mediterranean*)* of the Barcelona protocol concerning Specially Protected areas and Biological Diversity.

4. ECOSYSTEM CHANGES DUE TO ANTHROPOGENIC IMPACT

Anthropogenic activities which may cause direct loss and degradation of biodiversity include: fragmentation and loss of natural habitats, overexploitation of certain species, biological invasion, pollution, microbial contamination. Indirect threats are: the development of river basins and coastline, increase of human population, disturbance linked to leisure or industrial activities, exploitation of wild stocks, non recognition or under-assessment of marine diversity and natural resources in economic terms, weakness of legal systems and institutions, absence of adequate scientific knowledge and/or ineffective transmission of information (BIOMARE, workpackage 2, October 2001). The most often cited causes of local biodiversity degradation are "*eutrophication*" and "*organic matter (OM) enrichment*". Chemical pollutants may also reduce immunocompetence and increase high parasitic infestations.

In the Mediterranean the main stressors causing alterations of marine biodiversity and hence degradation of ecosystem quality that have been highlighted (EEA, 1999) are the following:

1. Eutrophication arising from agriculture, urban and tourist development.
2. Industrial and oil pollution,
3. Fishing, exploitation of living resources and mariculture.
4. Biological invasions through shipping and aquaculture

Microbial contamination

Eutrophication arising from agriculture, urban and tourist development has led to serious degradation of all biological components of the marine ecosystem including microbial contamination. Microbial contamination is mainly related to urban wastewater discharges and represents a potential risk to humans. Another sourse of contamination is through the consumption of poisoned shellfish. The situation has only partly been mitigated by building urban wastewater treatment plants in the Mediterranean countries along the coast. The demand from tourism for good bathing water quality has also pushed other countries into paying increasing attention to this problem. Nevertheless, about 90 % of municipal sewage is still untreated (EEA, 1999).

Industrial pollution

The impact of industrial/chemical pollution, along with that of eutrophication, on the biological diversity, are those most studied at all scales (spatial, temporal). The impact of the two stressors (complex of stressors) are alike in most cases and are therefore not easily discernible at most levels with the exception of the organismic level (biological effects). A good example of this complex of stressors is the change of the ecological quality in the *Posidonia oceanica* meadows. *Posidonia* is a seagrass species endemic to the Mediterranean, of major importance for marine biodiversity due to the high number of species finding food and shelter in its meadows. These meadows show at present alarming signs of degradation, especially in the northern parts of the Mediterranean. In the Liguria Sea nearly 30% of their original surface area has been lost in the 60s, during the period of rapid urban and industrial development along the Ligurian coast (Peirano and Bianchi, 1997). Another case is that of the red coral *Corallium rubrum* that is heavily exploited. However, its degradation is also attributed to reduction in water transparency due to pollution and turbidity.

Oil pollution

After a spill of a contaminant in Valencia, in early July 1990, hundreds of dead dolphins were washed up along the Spanish, French and Italian coasts, as well as on North African shores. During the summer of 1991, several hundred dead and dying dolphins were washed up on the beaches of southern Italy and Greece. Although pathogens clearly triggered some of these deaths, and epidemics have been known to occur in wild marine mammal populations, the immuno-suppressive effects of contaminants may have contributed to the severity of these incidents, perhaps by facilitating the spread of infection. This, and the additional chronic effects of organochlorines, could hinder, or even prevent, recovery of individuals from pathogenic disease.

Fishing and mariculture

The increase of the number of fish-farms (biological and chemical stressor) modifies local marine biodiversity in the southern Mediterranean Sea.

Fishing has increased by about 12 % in the past decade, with high exploitation of both bottom-living (demersal) and big pelagic (tuna and swordfish) stocks. Over-exploitation has led to a serious decline in the red coral (*Corallium rubrum)*, the date mussel *(Lithophaga lithophaga*) and many other invertebrates.

Biological invasions

The biological pollution represented by exotic species (also cited as alien and invasive) represent a growing problem due to the unexpected and harmful impacts they may cause to the environment, indigenous fauna, economy and human health.

The Mediterranean, open to the Atlantic, Pontic and Erythrean biota, is prone to invasions, particularly in its eastern basin. The oldest vector of introduction is the transportation of fouling biota, sessile and adherent, on ship hulls. Many cosmopolitan members of the fouling community are quite possibly older introductions into the Mediterranean while recent records are also attributed to introductions via ballast tanks. Transport and transplantation of commercially important exotic oysters has resulted in numerous unintentional introductions of pathogens, parasites and pest species. However, the greatest influx of invaders resulted from the opening of the Suez Canal in 1869 that allowed entry of Indo-Pacific and Erythrean biota, the so called Lessepsian immigrants (Por, 1978). Already some Erythrean invaders spread as far west as Malta and Sicily; if global warming were to affect the Mediterranean sea water temperature, tropical invasive species would gain a distinct advantage over the native fauna.

5. CURRENT FRAMEWORKS FOR THE DEVELOPMENT OF ENVIRONMENTAL INDICATORS

There are many frameworks within which indicators and potential indicators could be developed and used. World-wide several frameworks such as the “Pressure-State-Response” (PSR) and the “Sustainable Development” (SD) ones have been proposed for the design and organization of indicators. FAO[1] has outlined the guidelines of the process to be followed in order to establish (design, select, develop, implement, test) a Sustainable Development Reference System (SDRS) at national or regional level (FAO, 1999).

[1] FAO (the UN Food and Agriculture Organization)

The OECD[2] work on environmental indicators is carried out in close co-operation with OECD Member countries. It has led to:

- Agreement by Member countries to use the (PSR) model as a common framework;
- Identification and definition of a core set of environmental indicators supplemented with sectoral sets of indicators, based on their policy relevance, analytical soundness and measurability;
- Measurement and publication of these indicators for Member countries.

To assess the main challenges and problems for the marine and coastal waters in Europe, the Inter-Regional Forum Working Group on Indicators has framed the information needs, following the general conceptual assessment framework of the EEA, known as the DPSIR approach (Driving Forces, Pressures, States, Impacts and Responses) –see figure 2

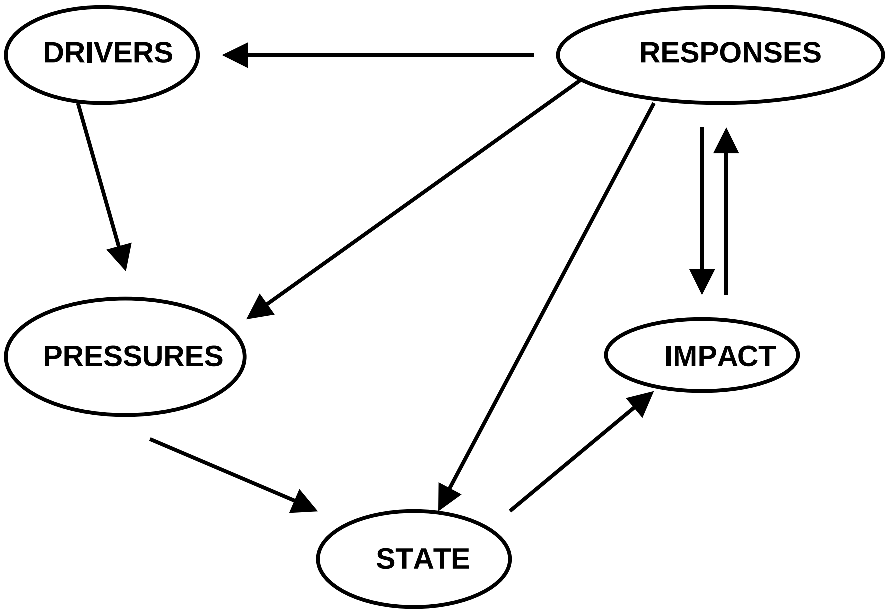

Figure 2. The DPSIR Framework for reporting on Environmental issues. Source: EEA, 1999b.

Driving forces describe the developments in human activities and economic sectors playing a key role in driving environmental change. ***Pressures*** describe direct stresses on the environment such as emissions to water, total input of substances to the coastal zone. ***State*** describes environmental (in this case biological) variables which characterise the conditions of marine waters and coastal zones. ***Impacts*** describe the changes in ecosystems. Due to the resilience of the ecosystem, changes in the environmental pressures do not always result in changes within the ecosystem. Moreover, changes in the state of the environment are so gradual that changes in the system are difficult to identify and often there is a time lag before changes become visible. The ***responses*** of policies can be defined specific in terms of measures affecting driving forces, pressures, state and impact or more generic like the adoption of integrated coastal zone management or the ecosystem approach in fisheries.

The Water Framework Directive (WFD)
A combined approach for the water protection at EU level is foreseen, with the implementation of the Water Framework Directive. Surface water, ground water and

[2] OECD: Organization for Economic Co-operation and Development

protection zones are the main environmental objectives of the WFD; the ecological status of these sectors is derived from the definition of "Eco-types" for the water elements, from 5 classes of ecological quality and from reference conditions.

Box 1. The Water Framework Directive (source directive 2000/60/EC)

The purpose of this Directive is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater which among among others:

a) *prevents further deterioration and protects and enhances the status of aquatic ecosystems and, with regard to their water needs, terrestrial ecosystems and wetlands directly depending on the aquatic ecosystems;*
b) aims at enhanced protection and improvement of the aquatic environment inter alia through specific measures for the progressive reduction of discharges, emissions and losses of priority substances and the cessation or phasing-out of discharges, emissions and losses of the priority hazardous substances;

……

and thereby contributes to:

– ………
– *the protection of territorial and marine waters, and*
– *achieving the objectives of relevant international agreements, including those which aim to prevent and eliminate pollution of the marine environment, by Community action under Article 16(3) to cease or phase out discharges, emissions and losses of priority hazardous substances, with the ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances.*

Selecting final indicators and their reference points

Indicators are especially important tools for accountability and transparency. This requires that they are limited in number, relevant, responsive, simple and policy-related.

Selecting the appropriate framework and determining dimensions, criteria, objectives and possible indicators and reference points, there may still be a large number of potential indicators that could be used. Once the indicators have been selected and agreed upon, the use of standardised methodologies and specifications for indicators and reference points will help to provide a sound technical foundation for a framework. They also help to ensure that comparisons within and between similar ecosystems are consistent through time. They need to be well documented and their applications widely understood and the use of a methodology sheet is recommended. The methodology sheets should, as far as possible, identify the data needs, method of analysis, and the frequency with which the indicator should be updated.

Indicators are generally developed from data that are already available, e.g. in institutional databases and industry records. However, there may be areas where criteria and objectives have been developed but there is no reliable data to calculate indicators and evaluate progress against the objectives. Where such deficiencies exist, an effort has to be made to collect data for a minimum number of indicators which will then be used to assess the ecological status of a given area.

6. PROGRESS IN DEVELOPMENT OF ECOLOGICAL QUALITY BIOLOGICAL INDICATORS FOR MARINE AND COASTAL WATERS

Environmental and in particular ecological quality indicators are being developed at a national level by many countries as part of their international obligations such as those under Agenda 21 and OECD reviews. However, most countries focus on chemical parameters. Some countries consider chlorophyll concentrations to be a useful indicator. It seems that biological indicators will need to be more focused upon in time. In Australia among 61 environmental indicators recommended for reporting the state of the environment (Ward et al, 1998), 3 relate to cited species, 9 to habitat extent and 17 to habitat quality (Table 4).

Table 4.: Key Indicators: Condition (C), Pressure (P) or Response (R) for Australian waters
Source : Ward et al, 1998

Class 1: Cited species/taxa	**Class 3: Habitat Quality**
1.1marine species rare, endangered or threatened R 1.2 protected species populations C 1.3 seabird populations C **Class 2: Habitat Extent** 2.1 algal bed area C 2.2 beach and dune area C 2.3 coral reef area C 2.4 dune vegetation C 2.5 intertidal reef area C 2.6 intertidal sand/mudflat area C 2.7 mangrove area C 2.8 saltmarsh area C 2.9 seagrass area C	3.1 algal bed species C 3.2 algal blooms P 3.3 beach species C 3.4 coral reef species C 3.5 dune species C 3.6 fish populations C 3.7 intertidal reefs species C 3.8 intertidal sand/mudflat species C 3.9 islands and cays species C 3.10 mangrove species C 3.11 pest numbers P 3.12 saltmarsh species C 3.13 seamount species C 3.14 seagrass species C 3.15 species outbreaks P 3.16 subtidal sand/mudflat species C 3.17 chlorophyll concentrations C

In the North Sea, measurement of Ecological Quality (EcoQ) has focused on the state of benthic communities based on the two community attributes: species diversity, and community structure and functioning (Table 5).

Table 5: Proposed indicators in the North Sea (after De Boer et al., 2001).

Species diversity	**Community structure and function**
Species diversity (Shannon-Wiener H')	r/K ratio as calculated through ABC method and W-statistic
Density of fragile, vulnerable species	Density of opportunistic species
Shell scars in *Arctica islandica*	VDS Index in female *Nucella lapillus*

In Sweden, the scale used to classify current conditions for the Kattegat/Skagerrak is based on an interpretation and integration of the biological structures on and within the bottom sediments (Pearson and Rosenberg, 1978), and on the depth of the oxidized sediment layer, which together yield a Benthic Habitat Quality (BHQ) Index. In the Baltic Sea, the assessment of conditions is based on a combined index (AAB) of species diversity, abundance and biomass of bottom living animals (Anonymous, 2000).

Some initiatives to develop biological indicators are noted in HELCOM[1] but others as those being developed within OSPAR[2] are also noted. OSPAR considered in the context of the development of ecological quality objectives (EcoQOs) major ecosystem components: **Plankton, Zoobenthos, Fish, Habitats, Birds and Mammals**.

At the European level, the development of biological indicators, as a tool for the protection of biological diversity of coastal and marine ecosystems has been advanced through the implementation of the HABITATS[3] directive, the ecological parts of the WFD[4], the ICZM[5], the Bathing Waters Directive and others. Moreover, the European Commission is funding several initiatives such as the European Platform for Biodiversity Research Strategy (EPBRS), BIOMARE (http://www.biomareweb.org) and Marble Conference in the framework of which the development of marine biodiversity indicators are key issues. At present the following indicators are being discussed within BIOMARE: *a)indicators of environmental change, b)keystone invasive and engineer species, and c)genetic and molecular biodiversity indicators*

Rec97ently a reform of the Common Fisheries Policy (CFP) so as to incorporate the ecosystem approach has been dictated by the loss of biological diversity due to fishing and aquaculture.

The biological quality elements for the definitions of ecological status in coastal waters as defined in WFD (EEC, 2000) are : a)*Composition, abundance and biomass of phytoplankton; b)Composition and abundance of other aquatic flora and c) Composition and abundance of benthic invertebrate fauna*. In particular, three quality classes, high, good and moderate can be assigned on the basis of the above elements as in Table 6.

As indicated above considering the Water Framework Directive, state of the environment information about European marine waters will build on the progress made in monitoring and assessments and indicator developments through the Conventions/Action Programmes and collaborating through the Inter Regional Forum Working Group on Indicators. The Inter-Regional Forum Working Group, following the general conceptual DPSIR framework of the EEA has identified and proposed for further development the potential core set of biological indicators presented in Table 7.

The development of indicators at EU level has been speeded up after the European Council in Cardiff in the summer 1998 and the activities in relation to integration of environmental concerns in relation to environmental policies.

[1] HELCOM: Helsinki Commission, Baltic Marine Environment Protection Commission
[2] OSPAR: Oslo-Paris Convention on the protection of Marine Environment of the North- East Atlantic
[3] HABITATS: refers to the Natura 2000 habitat types
[4] WFD: Water Framework Directive
[5] ICZM: Integrated Coastal Zone Management

Table 6: Definition of three quality classes of coastal waters based on phytoplankton, phytobenthos, zoobenthos (source WFD, Annex V, §1.2.4)

High status		Good status	Moderate status
Phytoplankton	The composition and abundance of phytoplanktonic taxa are consistent with undisturbed conditions. The average phytoplankton biomass is consistent with the type-specific physicochemical conditions and is not such as to significantly alter the type specific transparency conditions. Planktonic blooms occur at a frequency and intensity which is consistent with the type specific physicochemical conditions.	The composition and abundance of phytoplanktonic taxa show slight signs of disturbance. There are slight changes in biomass compared to type-specific conditions. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the quality of the water. A slight increase in the frequency and intensity of the type specific planktonic blooms may occur.	The composition and abundance of planktonic taxa show signs of moderate disturbance. Algal biomass is substantially outside the range associated with type specific conditions, and is such as to impact upon other biological quality elements. A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months.
Macroalgae and angiosperms	All disturbance sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present. The levels of macroalgal cover and angiosperm abundance are consistent with undisturbed conditions.	Most disturbance sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are present. The level of macroalgal cover and angiosperm abundance show slight signs of disturbance.	A moderate number of the disturbance sensitive macroalgal and angiosperm taxa associated with undisturbed conditions are absent. Macroalgal cover and angiosperm abundance is moderately disturbed and may be such as to result in an undesirable disturbance to the balance of organisms present in the water body.
Benthic invertebrate fauna	The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions. All the disturbance sensitive taxa associated with undisturbed conditions are present.	The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type specific conditions Most of the sensitive taxa of the type specific communities are present.	The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with the type specific conditions. Taxa indicative of pollution are present Many of the sensitive taxa of the type specific communities are absent

Table 7. Potential core set of indicators for the headline indicator issue Fragile ecosystems: marine and coastal waters (source EEA, 2001)

DPSIR	**Ecological quality**
	Plankton
S	*Occurrence of nuisance algae*
S	*Species composition*
S	*Diversity*
	Fish (non commercial species)
S	*Biomass of threatened species*
	Zoobenthos
S	*Community species diversity*
S	*Community structure and function*
	Habitats
S	*Surface versus potential surface*
	Marine mammals
S	*Biodiversity /numbers of threatened species*
	Marine birds
S	*Biodiversity/numbers of affected species*

7. INDICATORS BASED ON THE BIOLOGICAL ELEMENT: FROM GENE TO ECOSYSTEM

On investigating causal relationships between stressors and ecosystem health (impact on biological element), a great number of biological and ecological indicators have been developed as potential tools to trace biodiversity changes from molecular (gene) to ecosystem level. Biological indicators can be defined as multiple measures of organism health to environmental stressors that include several levels of biological organization and time scales of response.

The biological indicators that are in use today may range from biochemical to cellular and physiological level, called biomarkers, and from organism to ecosystem level termed bioindicators. A biomarker is defined as "a change induced by a contaminant in the molecular, biochemical or cellular components of a process, structure or function, that can be measured in a biological system'' (NRC, 1989).

Direct measurment of the presence of contaminants in the tissues of selected organisms, the so called biomonitors, has been the subject of long-term monitoring in the Mediterranean (as well as in other regional Seas). An important factor to be considered when establishing a biomonitoring system is the organism to be used. Criteria for selecting biomonitors have been proposed by several investigators, including spatial and temporal abundance, ease of sampling and range of detectable biological responses (summarized by Phillips and Rainbow, 1994). The range of marine organisms used by different researches is wide (including algae, invertebrates and vertebrates). Oysters, mussels and other taxa have been mostly used to monitor the water column levels of many chemicals, and represent an early warning device to detect the spread of unpredicted residues into otherwise uncontaminated areas. These have been used, for example, for detection of trace metals and organochlorines (Denton and Burdon-Jones, 1981; Phillips, 1985).

From a long list of proposed appropriate species (UNEP, 1981) each Mediterranean country has established its own set of organisms in the frame of the MEDPOL project as well as for the needs of national monitoring. The algae *Ulva* spp., *Enteromorpha* spp. *Posidonia oceanica* and benthic invertebrates *Mytilus galloprovincialis*, *Capitella* spp., *Malacoceros fuliginosa*, *Corbula gibba*, etc... are among those most often cited.

The presentation of indicators and rationale for using them as well as the linkage among the different levels are presented at the different levels of biological organization as below. Unfortunately, the links between biochemical and ecological levels are today still difficult to establish formally.

- At the gene level: molecular biomarkers to detect genetic changes induced at the organism and/or population level
- At the organismic level: biomarkers to measure biological effects: biochemical to physiological ↔ Linkage to molecular level
- At the population level: includes population genetics to detect stress, morphological changes and changes in population dynamics. ↔ Linkage to molecular and ecosystem levels
- At the community level: community composition, structure and function- a variety or a combination of ecological indicators ↔ Linkage to population level
- At the habitat level: monitoring of habitats of key species ↔ Linkage to population and community levels. Includes monitoring of birds and mammals as well as of pristine ecosystems like Specially Protected Areas.

Stressor effects on organisms, populations, communities and ecosystems, though measured at different time scales since the initiation of a stress may have a high ecological relevance. Yet, in most cases they cannot represent early signs of human pressures on the biological diversity of the ecosystem. However, several biomarkers are able to give the first warning of biodiversity threat.

At the gene level

These new simpler, faster short-cut largely laboratory techniques of monitoring are not meant to compete with or replace techniques that use population community and whole ecosystem responses (Munawar et al, 1989). The most basic level of biological responses is, perhaps, the molecular level, that of mRNA-level gene-expression. Changes in gene expression whether caused by a single gene mutation or a complex of multigene effects lie at the heart of regulatory mechanisms that control cell biology. Comparison of gene expression in different cell types, the onset of expression of gene-systems which are induced or suppressed by environmental cues, including levels of different pollutants, may offer the earliest biomonitoring markers (Wells, 1999).

The basic theory behind recent studies is that organismic response to environmental pollution entails, at least in part, *de-novo* transcription of messenger RNA (mRNA) (leading to protein synthesis). Research effort is focusing on testing the feasibility of the suggested approach for exposing markers based on alterations in gene expression. Polymerase Chain Reaction (PCR) is nowadays the standard tool for separating and cloning individual mRNAs. Differential Display Polymerase Chain Reaction (DD-PCR) provides for the detection of minute alterations in gene expression without requiring prior knowledge of responsive genes (Mokady and Sultan, 1998).

Research is designed in a way that would expose relevant markers for biomonitoring the coastal marine environment. In order to accomplish this objective, the obtained fragments (representing expressed genes) are screened for validity and sequenced, so that diagnostic PCR-primers could be designed for them. Identification of mRNA composition of cells, tagging and comparison with sequences in data banks and finally their cloning and usage as probes to isolate genes from genetic material or genomic libraries have been made possible by PCR analysis and nowadays (Liang and Parde, 1992) are steps for the development of ‘diagnostic kits’.

Research has taken a first step toward the development of an 'environmental diagnostic kit', based on multiple diagnostic fragments. To be useful, such a kit must be based on fragments obtained from an abundant biomonitor, reflecting alterations in gene expression in response to a wide range of types and levels of pollution and other environmental disturbances (Sultan et al., 2000).

In the Spanish littoral zone work is focusing on molecular biomarkers such as induction of biotransforming enzymes or some particular isoenzymes as early warning signals for ecological distress Higher activity of detoxifying and antioxitative enzymes in bivalves and fish Oxidative DNA damages assessed by High Performance Liquid Chromatography (HPLC).and gene expression using as specific probes the corresponding genes isolated by PCR (Barea, 1996). In Israel coastal marine bivalves are used in studies for the development of a gene –expression based marine biomonitoring system (Mokady and Sultan, 1998; Sultan et al., 2000).

Work in Mediterranean seems to be in its early stages exploring the possibility to develop a novel type of biomonitoring system focused at this level of biological response. The sensitivity of DD-PCR, enabling the visualization of very early responses, holds the promise for establishing not only a monitoring/assessment system, but rather a warning system with the capacity to prioritise different environmental problems according to their severity (Sultan et al., 2000). Thus, catastrophic events may be prevented rather than documented

At the organismic level

Biomarkers

Modern environmental toxicology has gradually combined the studies that are based on estimates of residue levels in bioindicator organisms with a new approach that relates the responses of an organism, population or natural community to chemical stressors in the environment, that is the development of biomarkers. The evaluation of a biomarker in bioindicator organisms that are sampled in one or more areas suspected of contamination and compared with organisms from a control area, enables the potential danger, to a community or communities, to be assessed (McCarthy and Shugart, 1990; Fossi, 1994). Up-to-date a number of advantages and limitations have been recording for the use of biomarkers.

Among the advantages the following can be assigned:

- Molecular, biochemical or cellular events tend to be more sensitive, less variable, more highly conserved between species, and often easier to measure than stress indices commonly examined at the organismic level.
- Molecular and biochemical alterations are the first detectable quantifiable responses to environmental changes.
- Molecular and biochemical markers can serve as indicators for both exposure and effects in organisms.

However it must be cited that:

- Age, diet, environmental factors, seasonal variation, and reproductive cycle may alter a number of structural states representing normality and could be potentially confounding issues in attempts to use morphological criteria as biomarkers of effect.
- Overlap between anticipated toxic state and some aspects of the range of normal morphology might exist.
- It is difficult to relate biochemical responses to the health of the organism and to adverse effects on the population, the type of information that is often the bottom-line in environmental monitoring. This problem can be overcome, however, by selecting biomarkers which detect cellular and biochemical events which are intimately involved in protecting and defending the cell from environmental insults.

1. Proteins and Enzymes

Different proteins and enzymes have been explored as potential biomarkers for a variety of different organisms (Huggett et al., 1992). The most frequently used techniques for analyzing protein levels within organisms involve metabolic labeling and specific antibody or cDNA probes. Antibodies appear to be the most promising probes for biomarkers and their use can be greatly increased if monoclonal antibodies are produced and to be used for detecting the same proteins in a large variety of different organisms. In the analysis of enzyme activity, enzyme assays can be run using various substrates to determine the rate of conversion of the substrate to its final product.

1.1. MFOs: Cytochrome P-450 dependent mixed function oxidases

Rationale: Cytochrome P-450 monooxygenases are a protein family involved in the biotransformation of organic chemicals, resulting in molecular changes (either their activation to toxic metabolites or their inactivation). The induction of P-450 can serve as a highly sensitive marker of an organism's toxic burden, when the organism has been exposed to chemical inducers in the environment (Rice et al., 1994).

Advantages: These enzymes are present in a variety of tissues (e.g. liver, gonads, kidneys, intestines, gills, heart). Increased P-450 enzyme activity generally results in the increased synthesis of mRNA and increased production of the enzyme protein. In a concept it is possible to study the activity of the P-450 enzymes, the amount of protein and the amount of mRNA in the cells but these procedures are expensive, labor intensive and require laboratoriess that are properly equipped and personnel with expertise in biochemistry.

Limitations: Few studies have been done with fish in order to evaluate the sensitivity of the induction cascade for this enzyme (Forlin et al., 1994). However enzyme's potential as a biomarker is somewhat limited at present. Similarly, the potential use of monooxygenase activity in molluscs or crustaceans for analyzing environmental chemical exposures seems to be little at present.

1.2. Stress proteins

Rationale: Stress proteins is a group of proteins whose synthesis is induced by a wide variety of physical conditions and chemical agents. There is an assumption that some of these proteins protect the cell from damage resulting from environmental perturbations. Others are involved in the regulation of various genes.

Advantages: Stress proteins make ideal candidates as biomarkers for environmental contamination since they are: a) part of the cellular protective response, b) induced by a wide variety of environmental stressors, c) highly conserved in all organisms from bacteria to man (many cDNA probes and antibodies can be used across phyla) and d) much is known about multiple levels of regulation of stress proteins

1.2.1. Heat shock proteins

Rationale: Heat shock proteins (hsp) are induced by a wide variety of stressors (Sanders 1993). There are five types of heat shock proteins: hsp90, hsp70, hsp60, hsp20-30 and ubiquitin. Of these, only three (hsp70, hsp60 and ubiquitin) have been potential as valuable biomarkers. The hsp70 acts to stabilize or solubilize target proteins and serves a "chapherone" function by helping newly synthesized secretory and organellular proteins translocated across the membrane (Hightower, 1991). The hsp60 facilitates translocation and assembly of oligomeric proteins in mitochondria (Hendrick and Hartl, 1993). Both hsp70 and hsp60 are highly conserved between species and are greatly increased in quantity under stressful conditions, making them ideal as stress indicators in organisms.

Advantages: Ubiquitin is an excellent biomarker because of it targets denatured proteins for degradation and removal.

Limitations: Its small size requires extremely sensitive equipment for detection. Thus, some work must be done before its full potential as a biomarker is realized.

1.3. Glucose regulated proteins

Rationale: Glucose regulated proteins (grps) are involved in the cellular responses to glucose and oxygen deprivation.

Limitations: Little is known about the induction of synthesis of grps. Their use as general stress indicators is limited but their function makes them ideal as specific response biomarkers.

1.4. Heme oxygenase

Rationale: Metals, sodium arsenite, thiol-reactive agents and stressors causing oxidative damage induce heme oxygenase. Its function is to cleave heme to form biliverdin, which is subsequently reduced to bilirubin. Bilirubin protects cells from oxidative damage as a free radical scavenger. This is a good biomarker for looking at stressor specific cellular responses.

1.5. Metallothionein

Rationale: Metallothionein is a protein that is induced by trace metal exposure. It is a metal-binding protein that is involved in the sequestration and the metabolism of heavy metals in cells.

For more details see UNEP/RAMOGE, 1999.

Advantages: These proteins have much to offer as potential biomarkers. A large information base that concerns available methods for monitoring both changes in metallothionein synthesis and metal composition is available. Assay procedures for these proteins are sensitive and give evidence of induction at relatively low metal dosage levels.

Limitations: Before these proteins can be effectively used as biomarkers, the normal physiological levels and their regulation must be understood.

1.6. Antioxidants

Rationale: Antioxidants are induced by the production of oxyradicals in cells, as a result of oxidant mediated responses. There are several types of antioxidant enzymes that could be used as biomarkers for environmental contamination. These include: superoxide dismutases; catalases; peroxidases and glutathione reductase. Of these four, peroxidases appear to have the highest present potential as biomarkers, particularly in the context of oxidizing air pollutants.

1.7. Cholinesterase

Rationale: Cholinesterase (ChE) is widely used to estimate neurotoxic impacts of pollutants on the cellular level of marine organisms (Galgani et al., 1992). Inhibition of ChE activity has been suggested as a parameter to detect effects of organophosphates, carbamates, some heavy metals and surfactants (Escartin and Porte, 1997).

2. Other Potential Protein And Enzyme Biomarkers

There are many other protein and enzyme biomarkers that are being explored presently.

These include:

ATPases
Monoamine
NADPH cytochrome c reductase and other cytochromatic enzymes

Various hormones
Glutathione peroxidase and glutathione transferases
Heme biosynthetic pathway enzymes
Epoxide hydrolase and many others
Vitellogenin

3. *Immunological Biomarkers*

The response and functioning of the immune system is multifaceted. Consequently it is highly susceptible to alteration or inhibition by environmental stressors. Recent experiments correlate the decrease in immunocompetance in marine organisms to the PCB content in their fats (Stone, 1992) or to the pollution gradient (Secombe et al., 1991). There are many assays available for detection of contamination, but the ones used are often chosen based on the specific objective of the study, the available equipment, the experience and training, the length of the study and the number of tests being done. A number of advantages and limitations of using immunological indicators as biomarkers is cited in table 8.

Table 8. Advantages and limitations of using immunological indicators for assessing EcoQ

Advantages	Limitations
• Response occur even when chemicals' concentration is low	• Data cannot be extrapolated between species
• Provide evidence linking a toxicant to disease outbreak in fish	• Immune response is sometimes too broad to provide conclusive evidence
• Assays are quick an sensitive; simpler assays can be taken into the field as kits	• Difficult to know which test to use or what effects to test for
• Do not require sacrificing the organism	• Assays are specialized and require subjective interpretations
• Blood samples can be taken over a period of time to allow for long term evaluation of a toxin	• Complex assays are expensive
• Immune system is physiologically similar in most vertebrates therefore equipment/materials can be used on a variety of species	• Stressor must already be known
• There is a large literature base of immunotoxicology	• Immune system is highly sensitive to biotic and abiotic factors • Requires further confirmatory testing

Immunological indicators may be categorized into a three tier regime. Tier I consists of general screening of the immune system, tier II is a comprehensive evaluation of the immune system and tier III consists of host resistance studies.

Tier I: General Screening (nonspecific screening)

This level includes studies related to gross morphology or cellular conditions of the organs of the immune system. Examples involve measurements of the size and weight of the spleen, the volume of packed cells within a unit volume of blood (hematocrit), the volume of leukocytes per unit volume of whole blood (leukocrit), wound healing, macrophage phagocytosis, lysozyme activity, agglutination assay and graft rejection. These assays are quick, easy, and inexpensive but are sensitive to temperature, handling and crowding of the organisms.

Tier II: Comprehensive evaluation of immune system

This level involves a variety of assays which examine all the components of the immune system and include immune cell quantification, native immunoglobin quantification, surface markers, phagocytic index (cells involved in the initial immunal response) and plaque-forming cell assay. Another test is to measure the mitogenic response that is the index of factors stimulating proliferation of B or T lymphocytes. It is known that stressors will suppress the stimulatory ability of such factors causing the mitogenic index to drop. Assays of tier II are specific and sensitive. All compounds causing tier II effects have been found to cause tier I effects; therefore tier II tests should be used only when studying the mechanism of action of the toxicant.

Tier III: Host resistance studies

This level focuses on the resistance of the host to a stressor. Tests include mortality, noting the presence of and quantifying bacteria, viruses and parasites in the blood and tumour quantification measuring antigen uptake and performing specific antibody quantifications.

4. Histological Markers

Histopathology is a valuable discipline for determining effects on cells and tissues. Besides a series of molecular/biochemical changes caused by stressors, structural changes to cells may also occur. The cells are able to survive many types of injury by means of adaptive physiological response. Examples of such adaptations include hypertrophy, atrophy, increased lysosomal autophagy, ageing, neoplastic transformations and accumulation of materials. However, a study of the literature survey produced by Cantillo (1991) on Mussel Watch programs throughout the world shows that only 34 of the 1134 references contained histopathological studies. The studies on fish histological modification have mainly focussed on the detection of neoplastic transformation or foci development. Modifications of the lysosomal system such as its permeability, stability, size, proliferation and content have been utilized as biomarkers of effect. Some tests use cells in body fluids, for example blood, which can be used nondestructively, such as pathological changes in intracellular membranes of lysosomes. Lysosomal membrane damage appears to be a universal marker for effects of stresses in most if not all nucleated cells (Moore et al., 1994).

It is important to note that by relating biomarkers of cell injury to significant pathological consequences for the organism their diagnostic value and predictive capability for further damage at higher organisational levels will be strengthened (Moore and Simpson, 1992). A number of limitations and advantages is cited in table 9.

Table 9: Advantages and limitations of employing histological markers to assess EcoQs.

Advantages	**Limitations**
• Results from initial tests can lead to appropriate detailed analysis	• Need to know the normal appearance of organs and tissues
• A number of different organ systems can be analysed using the same organism	• Need to understand the normal fluctuations that occur in tissue during life cycle and time of year
• Rapid assessment of many potential sites of injury	• Must take into consideration diet and other factors influencing the organism
• Stressors attack specific cell types within a given region of an organ	• Assays have to be done carefully
• Potential predictive qualities (type of chemical, health of ecosystem)	• Examiner needs to be able to detect changes in tissue and organ

• Technological development have increased the resolution of the assays • Integrate net effects of biochemical and physiological changes • Address acute and chronic exposure	• Assays are only as good as the person using them

Yet, much antibody-based recognition tests for specific proteins (e.g. cytochomes P450, stress proteins) can now be applied directly to histogolical samples. This can provide useful information on the spatial distributions of such proteins in relation to stress-induced structural and organizational alterations in cells and tissues (Moore and Simpson, 1992).

Among the many potential indicators to be used for EcoQOs two have been proposed by UNEP/RAMOGE, 1999. These are: a) Mixed Function Oxygenase System (MFO), and b) Metallothionein Level (MT). For details see UNEP/RAMOGE, 1999

At the population level

Certain individuals within a population may be more vulnerable than others to this incipient toxicity due to their specific phenotypes, and disappear from the population long before others are affected. Sublethal levels of pollutants may thus be associated with the loss of genetic diversity within a population subjected to pollution even though the population as a whole, at least over the short term, is able to survive and thrive (Street and Montagna, 1996).

During the past three decades, the scientific community and regulatory agencies have become increasingly aware of the long-term impact of environmental stressors on the sustainability of ecosystems, on the depletion of biodiversity and genetic variability in natural populations, and the extinction of species. Stressors can directly or indirectly induce changes in genetic variability and allele frequencies of populations that result from induced mutations, population bottlenecks, and selection caused directly or indirectly by contaminant exposure. Nevertheless, only a handful of studies have addressed the effects of chemical contamination on population genetics.

Contamination can affect the genetics of natural populations in two ways: genetic variability is increased by the appearance of new mutations, or overall genetic variability is decreased by population bottlenecks. Either of these effects can be accompanied by altered allele frequencies, perhaps resulting from selection at loci important for survival in polluted environments or from fixation of deleterious alleles. Reductions in overall genetic variability will be the most frequently observed effect (Bickham et al., 2000). Theoretical work provide that population from unpredictable environment, present a general reduction in genetic variability (Battaglia and Bisol, 1988, Cognetti and Maltagliati, 2000).

Population-genetic and evolutionary effects of contaminant exposure have recently attracted the attention of ecotoxicologists (Belfiore and Anderson, 1998, Cronin and Bickham, 1998, Depledge, 1994, Hebert and Luker, 1996). The capability now exists to detect genetic variability within and among populations by a variety of sensitive molecular procedures that allow sample sizes large enough to detect subtle changes in the genetic make-up of populations.

Detection of genetic variability is achieved through:

- allozyme analysis (medium sized organisms such as macrozoobenthos)
- diversity in the nuclear genome,

- sequence diversity in the mitochondrial genome (mtDNA) - sensitive to the genetic effects of population bottlenecks, effective genetic system for monitoring population declines.
- sequence characterization of the nuclear genome (randomly amplified polymorphic DNA (RAPD) technique used to screen for markers linked to functional loci open to selection and microsatellites detecting heritable mutations resulting from contaminant exposure and differences in overall levels of genetic varia)

Levels of genetic variability can also be altered by a variety of natural processes. It can be increased by gene flow, which is the effective exchange of migrant individuals among populations, interspecific hybridization, and mutations. Of these, migration is by far the most important at least for ecological (not evolutionary) time scales. Genetic variability can be reduced as a result of bottlenecks and selection resulting from natural processes like diseases, climatic changes, or weather patterns. In addition, seasonal variations and other patterns of population fluctuations, such as the cycles observed in some rodent and insect species, might alter levels of genetic variability. Work by Camili et al. (2001) proved the allozymic genetic divergence in bivalves in brackish and marine habitats due to superimposition of two main evolutionary forces: diverse selective regimes and different history of colonisation and/or geographical distribution.

Because population genetic changes are expected to be independent of the mechanisms of toxicity, and yet highly sensitive indicators of transgenerational effects, Bickham et al. 2000, propose that they should represent the ultimate biomarker of effect '*This is because genetic changes, especially the loss of genetic variability, might be permanent — once variability is lost, the population cannot recover to what it was prior to the environmental impact. Whereas population numbers can potentially recover to pre-bottleneck levels as a result of adaptation to the polluted environment or disappearance of the stressor, genetic diversity will only recover if the population survives for a very long time (assuming the absence of gene flow from other populations). This contrasts with other biomarkers of effect, which represent somatic effects on individuals, not permanent effects in populations'.*

However, works focusing directly on the effects of pollution on population genetics are very limited and mostly in theoretical level especially in the Mediterranean.

Stress impact at population level can be assessed through morphological studies for large animals.

Genetic diversity for small-sized organisms or cryptic species.

Population dynamics is also a means to assess impact on large animals (birds, mammals) and plants though the impact cannot be related directly to a specific stress factor.

Suggested Indicator: Populations of Key Species including protected ones

At the community level

In general marine communities respont to environmental stress by: a) reducing species diversity (fewer species), b) Regression to dominance by a few opportunistic species, c) Reduction in mean size of the dominating species (linkage to population level) and d) Elimination of species characteristic of higher trophic levels (Gray, 1979, 1989). The different effects originate from difference in morphology, ecology, and reproductive strategy among the species. Two main groups of species have been thus designated. The vulnerable, low reproducing species following a so called k-strategy and the fast reproducing ones the so called opportunists which follow the r-strategy. The differences between the two categories, summarised by Hotmann (1999), are given in Table 10.

Following the above, community diversity as a response to any stress factor can be measured at different levels of organization. These include:

- species variety per taxon (polychaeta, mollusca, crustacea…) or for all taxa
- presence and/or abundance of ecologically meaningful species such as endemics, exotics, sensitive, opportunistics
- relative abundance of functional groups (Suspension feeders, Predators, Taxa indicating environmental disturbance)
- various community indices

Table 10. Differences in characteristics between opportunistic species (r-strategy) and sensitive species (K-strategy) (from Holtmann 1999).

	r-strategy	**K-strategy**
Environmental stability	variable	constant
Mortality	often catastrophic	density dependent
Population size	variable in time	relatively stable
Inter and intra specific competition	often weak	intensive
growth	fast	slow
Reproduction	at an early stage	at a relatively older age
Body size	small	large
Age	often < 1 year	several years

The plankton diversity is essential to sustain diversity of fish, mammals and birds and also to respond to the spatial and temporal variability of the environmental constraints. However, its rapid changes, due to natural environmental conditions (hour of the day, currents, temperature etc) does not allow for reliable environmental assessment related to anthropogenic impact other than eutrophication. Thus, species variety (number of planktonic species in a given type of ecosystem) based on the entire spectrum of the plankton (phyto- and zoo-plankton) cannot be used as an indicator of ecosystem quality.

What can be used and is often used in planktonic studies is the dominance of few species-monospecific communities vs multispecies ones. In a given community under pollution usually there are some species that reach high abundance, most of them decrease significantly, while some other remain unaffected (Gray *et al.* 1988). In this line several dominance indices have been widely applied with success in planktonic data sets among which the ones suggested in this work are the following:

DOMINANCE INDEX: Dominance of opportunistic species/groups
OCCURRENCE OF NUISANCE SPECIES: Presence/outbreaks of nuisance taxa

Soft bottom benthic communities have been used in developing ecological parameters/indicators because benthic animals are mainly sedentary, have relatively long life-spans and exhibit different tolerances to stress. Thus zoobenthic community structure has been said and proved to be a reliable measure of ecosystem “health”. In this line, monitoring of benthic communities, although it may me time-consuming, has often been applied in environmental impact studies (fisheries, domestic/industrial effluent, dumping of solid waste etc).

A general evolutionary pattern of the marobenthic biocoenosis of the soft bottom substrate under the influence of a perturbation factor, (of anthropogenic origin) has been described world-wide, based on the work of Pearson and Rosenberg, 1978, and in the Mediterranean by Peres and Bellan (1973), Ros and Cardell (1991) and Salen-Picard (1981,1997)

The changes that a benthic community undergoes under the influence of the disturbance from an initial state of high diversity and richness in species and individuals is as follows:

1. A regression of the species strictly linked to the original conditions of the environment.
2. Certain tolerant species considered as pollution indicators tend to monopolise available space. A limited increase of diversity can be observed in this state. The biocoenosis structure remains recognisable even if degraded (subnormal zone).
3. A destruction of the biocoenosis is recorded; certain species exist and develop, apparently independently of each other. Species diversity decreases and becomes minimal (polluted zone).
4. The macrobenthos disappears (zone of maximal pollution).

A first step towards the interpretation of the impact of human pressures on the benthic communities would be to recognise the different benthic communities in the Mediterranean Sea. Simboura and Zenetos (in press) have revised the main soft bottom community types encountered in the Mediterranean, by adjusting the classical bionomic scheme of biocoenoses described by Peres and Picard (1964) to European typology (see table 11) that is considering both the main environmental (depth, type of substratum) and biotic factors (i.e.phytal cover). Thus, the term “community types” which includes the environmental aspect, is used in a broader sense very similar to that of “habitat”.

Table 11. Soft bottom community types proposed by Simboura and Zenetos (in press). Abbreviations used: VTC=Coastal Terrigenous muds; LEE=urythermal and Euryhaline biocoenosis (met in lagoons and estuaries); SFBC=biocoenosis of well-sorted sands; SFHN= fine surface sands; SGCF=coarse sands and fine gravels under the influence of bottom currents; SVMC=muddy sands in protected areas; AP=Photophilous algae biocoenosis. DC=Coastal detritus bottoms. C=Coralligenous.

Type of community Proposed	**Peres and Picard, 1964 Definition**	**Alternative Description**
Midlittoral sands		Midlittoral sands
Deltas	LEE	Brackish, deltaic ecosystems
Lagoons	LEE	Transitional lagoons
Muddy sands		Mixed sediment (shallow 30m or deeper 30-100m)
Muddy sands with phytal cover		In or close to phytal meadows of macroalgae or angiosperms (*Zostera, Posidonia, Caulerpa)*
Shallow muddy sands	SVMC	muddy sands in protected areas
Sandy muds	VTC	Sub-community of muddy bottoms with *Amphiura filiformis*
Shallow muds		Shallow muds (20m)
Deeper muds	VTC	muds deeper to 50m (typical VTC)
Shallow sands	SFBC, SFHN	Shallow sands (well sorted or very shallow sands)
Deeper coarse sands	SGCF	Coarse sands in high energy environments
Deeper Sands with detritus	DC	Deeper sands with biogenic fragments or Coastal detritic bottoms
Coralligenous	C	Deep, sciaphilic

In Conclusion, benthic communities are to be used as indicators for effects acting at the level of community diversity, structure and function. Species which fulfil important ecological functions should be given particular attention in a community context and indicator species for the impact of human activities including those threatened and declining are taken into account within this report. Based on the findings of the effects of human pressures on the benthos (zoobenthos, phytobenthos) diversity, structure and functioning, the following indicators are suggested to monitor the impact on the community level of biological diversity.

Main indicators

NUMBER OF BENTHIC SPECIES AND NUMBER OF EXOTIC SPECIES
PRESENCE AND COVERAGE OF BENTHIC MACROPHYTES
PRESENCE/ABUNDANCE OF SENSITIVE AND/OR OPPORTUNISTIC ZOOBENTHIC SPECIES/TAXA
COMMUNITY DIVERSITY (H) OF ZOOBENTHOS, PHYTOBENTHOS
BIOTIC INDEX: complex index incorporating ecological groups

Additional Indicators

LEGISLATION CONCERNING RARE, ENDANGERED OR THREATENED MARINE SPECIES
EVENNESS OF DISTRIBUTION
COMPARISON OF DOMINANCE CURVES (ABC, W-statistics)
LOG-NORMAL DISTRIBUTION
GEOMETRIC ABUNDANCE/SIZE CLASSES DISTRIBUTION
THE RATIO BETWEEN r- AND K- SELECTED SPECIES
INFAUNAL TROPHIC INDEX: based on the trophic status of the zoobenthos

It must be realised that the operational applicability of the above indicators for measuring Ecological Quality (EcoQ) of benthic communities is not without restrictions.

At the habitat level

The diversity and complexity of marine life can also be impacted by human pressure at broader scale than community; that of habitat [encompassing biological and abiotic (physical, geological) attributes] and that of the ecosystem. Marine biological diversity is dependent on the spatial scale adopted and on the measurement tools used. The vast number of species and genes, the taxonomic difficulties and the high cost of conducting detailed studies, even if only in coastal waters, dictates the use of surrogates to be used at higher levels. These include:

- *Remote sensing and mapping*: In shallow areas: Airborne - high resolution photographs and satellite imaging. In deeper areas by means of multibeam sonar and side scan sonar

- *Rapid Assessment Surveys*

The more remote the surrogate is from the target level of diversity i.e remote sensing to monitor a species-specific biotope, the greater the risk that the surrogate is not effective. In such cases Rapid Assessment Surveys (RAS) sould be conducted. These could be based either on airborne photographs or to visual monitoring of the habitat of the so called “key species”. *Visual monitoring* can be carried out by means of photo and video recording:

Transects by Snorkelling and/or Scuba diving. Image analyses has been used to map communities and bottom types in littoral lagoons in Corsica (Pasqualini et al., 1997). In European Waters remote sensing has been widely used in mapping the NATURA 2000 Sites (Habitas Directive).

- *Destructive sampling* : Quadrants by Snorkelling and/or Scuba diving

measuring a) cover (percentage of surface covered by vertical projection of species) and b) community structure. Community structure is examined in terms of vegetation layer (turf, encrusting layer and erect layer) and macroalgal functional groups (filamentous, corticated terete, articulated calcareous and crustasea), followed by typical analysis of species composition and abundance.
Bathymetric, acoustic and visual surveys can aid in mapping habitats and thus follow changes in benthic habitats. Destructive sampling is time consuming and needs the same expertise as the traditional sampling of soft bottoms.

Advantages: Acoustic methods seem very useful for inventories of habitats (cf. Port-Cros map). These methods should be more detailed in order to choose a certain number of them. Visual monitoring can also be very effective.

Limitations: the quality of ground-truthing although collaboration with other experts (physical oceanographers..) is possible. Lack of common protocols for deriving comparative results.
Turbidity in lagoonal ecosystems may be prohibitive for remote sensing.

SUGGESTED INDICATOR: CHANGES IN THE DISTRIBUTION AREA OF HABITAT TYPES

List of indicators

The table that follows (Table 12) includes the indicators suggested for assessment of the EcoQ, which will be described in detail in the following chaper. These have beed grouped as core set (the suggested ones) and additional (useful but second priority ones). However, in their description they are cited according to biological organization level they refer to.

Table 12: List of biological indicators *(For details see UNEP/RAMOGE, 1999)

A. Core set Indicators

1. Populations of Key Species including protected ones
2. Occurrence of Nuisance Algal Species
3. Number of Exotic Benthic Species
4. Number of Benthic Species
5. Presence and Coverage of Benthic Macrophytes
6. Presence/Abundance of Sensitive Zoobenthic Species/Taxa
7. Abundance of Opportunistic Species /Genera/Taxa
8. Community Diversity (H) of Zoobenthos, Phytobenthos
9. Biotic Index
10. Changes in the distribution area of Habitat Types

B. Additional Indicators

1. Mixed Function Oxygenase System (MFO)*
2. Metallothionein Level (MT)*
3. Legislation Concerning Rare, Endangered or Threatened Marine Species
4. Dominance Index: Dominance of Opportunistic Pelagic Species/Groups
5. Evenness of Distribution
6. Comparison of Dominance Curves

7. Log-Normal Distribution
8. Geometric Abundance/Size Classes Distribution
9. The Ratio Between R- and K- Selected Species
10. Infaunal Trophic Index: Based on the Trophic Status of the Zoobenthos

8. DESCRIPTION OF SUGGESTED INDICATORS

Populations of key species including protected ones

Definition: Status of populations of each species of: marine mammals, reptiles, seabirds, fish, invertebrates and plants as well as migratory seabirds that are the subject of various international and bilateral conventions and agreements such as:

- Action plan for the management of the Mediterranean monk seal *(Monachus monachus*). (UNEP-RAC/SPA, 1999a)
- Action plan for the conservation of Mediterranean marine turtles (Demetropoulos and Chadjichristophorou, 1995, UNEP RAC/SPA, 1998c, UNEP RAC/SPA, 1999b, 1999c).
- Action plan for the conservation of marine vegetation in the Mediterranean Sea (UNEP RAC/SPA. 1999d.

Rationale: Stress impact at population level can be assessed through morphological studies for large animals. Reduction in size is a well established response to environments stress. Moreover, monitoring of the population dynamics is expected to reflect reliably the effectiveness of management measures.

The endangered or threatened species listed by the Bern Convention should be all considered as key species (Table 13). In addition the species whose exploitation is regulated (Table 14) are included in this category. Morphological changes in sea-urchin populations as a response to environmental stress have been cited in the Ionia Sea (Pancucci et al., 1993). Population dynamics studies are focused on Key species such as the sponge *Eunicella singularis* (Skoufas et al., 1996), other sponges (Ben Mustapha and Abed, 2001), the turtle *Caretta caretta* (Jribi et al., 2001) etc.

A monitoring programme must include survey of sensitive populations. A few monitoring networks in Europe are using population studies for evaluating the marine environment health or the state of the marine biodiversity. A good example is that of the *Posidonia* Monitoring Network (RSP), set up in 1984. It is probably the older Mediterranean monitoring system using routinely a “key-species” as bioindicator. The exotic algae *Caulerpa toxifolia,* is also a good bioindicator, well studied in the framework of the MAP for the conservation of marine vegetation in the Mediterranean. It also highlights the usefulness of population dynamics in studying the invasion impacts. In 2001, as a consequence of the 1999 mass mortality event which occurred in the NW Mediterranean, a network based on measurements of the gorgonian vitality has been set up in France.

Advantages: Development of this indicator falls within the objectives of the CBD.

Many populations are monitored in the frame of Action plans, by NGO’s.

Limitations:

While there are protocols to monitor the populations of some “key species” there is no protocol for most of them.

It is difficult to establish an index receiving continous values depending on the degree of stress, so as to assess directly the impact to stress or response to a managerial strategy.

Legislation concerning rare, endangered or threatened marine species

Definition: The number of marine mammals, reptiles, birds, fish, invertebrates and plants (including relevant species of seagrasses or algae) in each of the relevant IUCN[1] (World Conservation Union) categories or International Conventions and the subject of State legislation. State obligations of Mediterranean Countries under international agreements include:

- The Convention on International Trade in Endangered Species (CITES).
- The Bern Convention the EU Birds and Habitats Directives which are being implemented in all the European countries. Thirteen marine Mediterranean species are listed among the strictly protected fauna under the EU Birds and HABITATS Directives and NATURA2000 and the Bern Convention.
- Action Plan for the Conservation of Cetaceans in the Mediterranean Sea. (UNEP/IUCN, 1994; UNEP RAC/SPA, 1998a)
- A special agreement under the Bonn Convention, made in 1996, for the Conservation of Small Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic area the so called ACCOBAMS).

The indicator is being developed as part of the CBD. The draft list of endangered or threatened species compiled by UNEP (1999) coincides with that of Annex II of the Specially Protected Areas protocol under the Barcelona Convention, revised in the Bern Convention, 1998. The main species and taxa to be covered are:

- plants — vascular; algae.
- invertebrates
- birds
- reptiles — sea snakes; turtles; crocodiles.
- fish.
- marine mammals — whales; dolphins and porpoises; seals; .

Advantages: Easy to apply provided there is good collaboration and information flow from state authorities and NGO's.

Limitations: The species/taxa to be included under this indicator will change as the various jurisdictions gather better information about the species and taxa of concern.

Number of benthic species

Definition: The number of benthic species encountered in a well defined community type.

Rationale: The number of species in a benthic community varies greatly with depth and sediment type. A typical trend exhibited within the Mediterranean is a significant decrease in species number with depth Figure 3

[1] Unternational Union for the Conservation of Nature

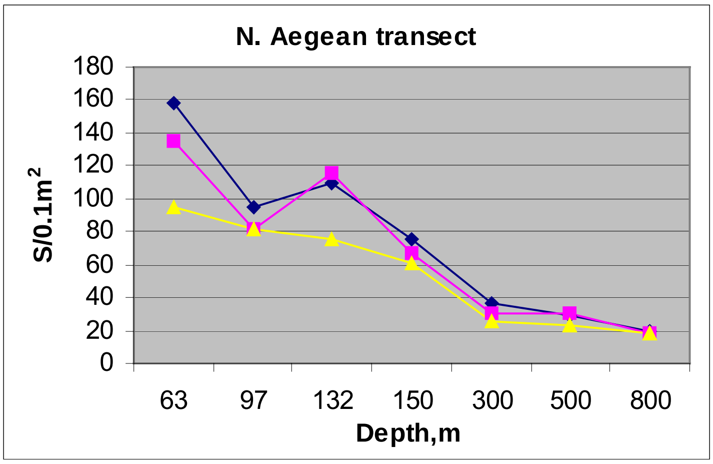

Figure 3. Trend in species variety with depth. Lines correspond to replicates. Source: NCMR, 2001

Sediment type is the second most significant factor influencing the species variety in a given biotope. In the two examples presented in Figures 4 it is depicted that different communities (benthic assemblages in certain sediment type/depth) hold different species numbers**.** The data are from two very well studied areas of the Aegean Sea which, being away from any land based pollution sources and thus unaffected from anthropogenic activities, serve as reference sites. In the graph, it is clear that species number per sampling unit is not dependent on season. However, it increases, the larger the sampling area is. Thus, from an average of 72,5 species /0.1m^2, it increases to 350 species/7m^2 in the silty sand site and from 23 species/0.1m^2, it reaches 128 species/7m^2 in the silty site respectively. On comparing the species number of a sampling unit with that of the average of either 10 samples (AVG S in figure) or of 70 samples (AVG/0.1m2 in the legend of figure), it appears that species number of a given unit (sediment surface area), can be an accurate measure of the state of environmental.

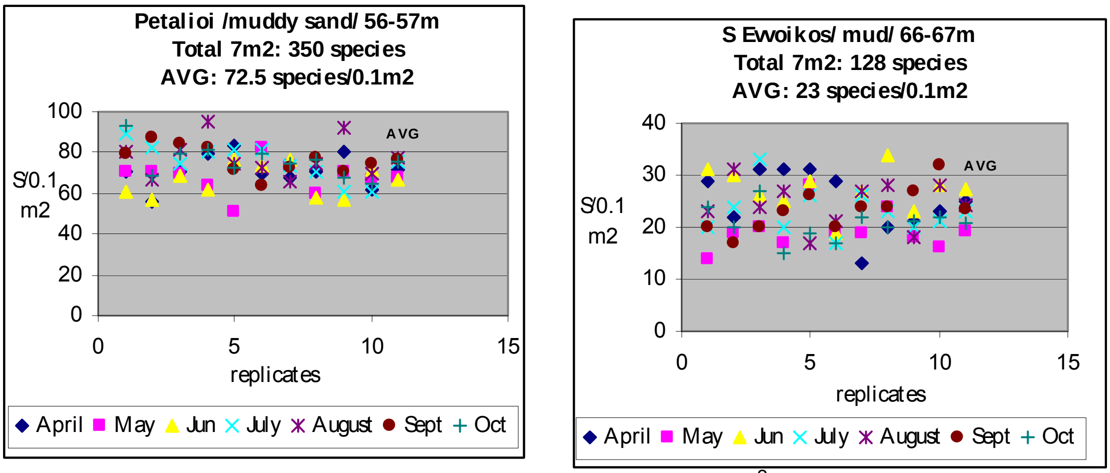

Figure 4: Variation in species number per sampling unit (0.1m^2) and monthly average (0.1m^2) in different habitats (depth, sediment type). Data from TRIBE project (NCMR 1997)

One of the central patterns in biodiversity, noted universally, is that the number of species increases with the area sampled. Data from the N Aegean Sea clearly show this increase with increasing sampling effort. In figure 5 it is demonstrated that the number of taxa discovered in an area is proportional to the sampling and taxonomic effort exerted. The number of taxa discovered in an open sea area of N Aegean increased from 340 (from a sampling surface 2.66 m^2) to 606 (at 12.14m^2).

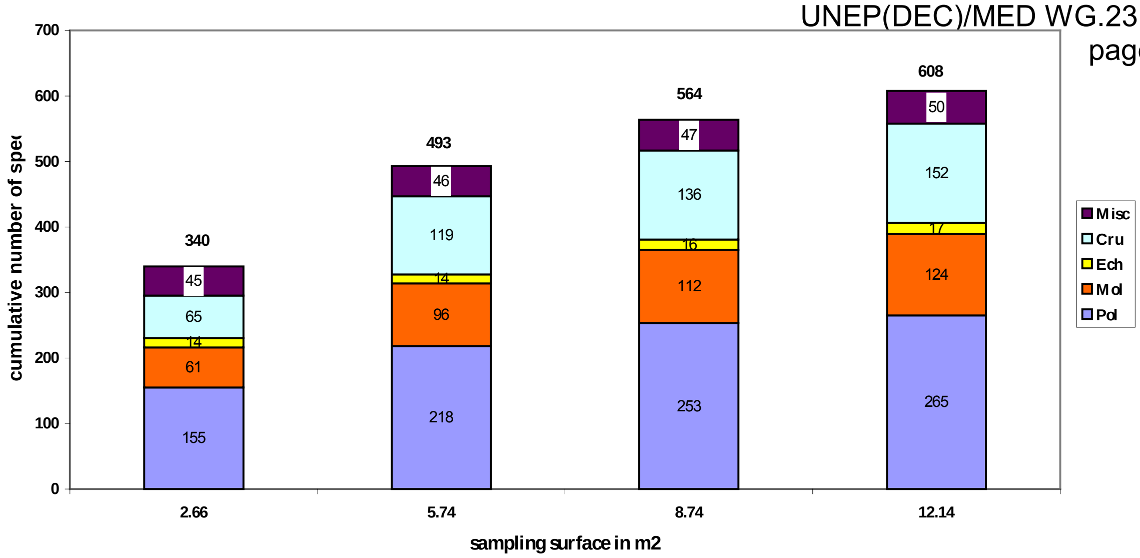

Figure 5: Trend in species variety with sampling surface sampled/analysed. Source: NCMR, 2001

An example of the effectiveness of S in ecological quality assessment is presented in Table 15. The reduction in species variety is directly correlated to the stressor. The data is from an impact study following an oil spill in an Aegean gulf. The trend is the same whether calculated on smaller sampling area (mean values) or bigger (pooled samples).

Table 15: Response of species variety to oil pollution in a given community type (shallow muddy sands). Source (NCMR, 2001).

	$0.05m^2$	$0.2m^2$
high: reference site	62.7	124.0
good	42.0	89.7
moderate: oil polluted	31.5	78.5
poor: oil polluted	21.3	35.5
bad: azoic	0	0

<u>Advantages</u>: The number of species (S) can be a reliable measure of environmental stress. Reference values (range of values) of S for “normal/undisturbed” communities should be developed for different community types (biotopes) to be used for quality assessment studies in disturbed ecosystems. These values however, can be different for different seas and regions. Deviation from reference values will then be indicative for the degree of environmental stress.

<u>Limitations</u>: Definition of S should apply

- to a well defined sampling unit (standard $0.1m^2$)
- to samples collected with the same gear (standard grab $0.1m^2$, mesh sieve 0.5mm)
- at the same community type (depth range and sediment type).
- If identification is being done at the same taxonomic level (4 major groups or all groups)

Table 13.
LIST OF ENDANGERED OR THREATENED MARINE AND FRESH WATER SPECIES IN THE MEDITERRANEAN **(Annex II** of the Protocol concerning Specially Protected areas and Biological Diversity in the Mediterranean Sea adopted in the Barcelona Convention in 1996); revised in the Bern Convention, 1998.

Magnollophyta	**Porifera**	**Fish**	**Mammalia**
Posidonia oceanica	*Asbestopluma hypogea*	*Acipenser naccarii*	*Balaenoptera acutorostrata*
Zostera marina	*Aplysina cavernicola*	*Acipenser sturio*	*Balaenoptera borealis*
Zostera noltii	*Axinella cannabina*	*Aphanius fasciatus*	*Balaenoptera physalus*
	Axinella polypoides	*Aphanius iberus*	*Delphinus delphis*
	Geodia cydonium	*Carcharodon carcharias*	*Eubalaena glacialis*
	Ircinia foetida	*Cetorhinus maximus*	*Globicephala melas*
Chlorophyta	*Ircinia pipetta*	*Hippocampus hippocampus*	*Grampus griseus*
Caulerpa ollivieri	*Petrobiona massiliana*	*Hippocampus ramulosus*	*Kogia simus*
	Spongia agaricina	*Huso huso*	*Megaptera novaeangliae*
	Spongia officinalis	*Lethenteron zanandreai*	*Mesoplodon densirostris*
Phaeophyta	*Spongia zimocca*	*Mobula mobula*	*Monachus monachus*
Cystoseira amentacea	*Tethya* sp. plur.	*Pomatoschistus canestrinii*	*Orcinus orca*
Cystoseira mediterranea		*Pomatoschistus tortonesei*	*Phocoena phocoena*
Cystoseira sedoides		*Valencia hispanica*	*Physeter macrocephalus*
Cystoseira spinosa		*Valencia letourneuxi*	*Pseudorca crassidens*
Cystoseira zosteroides			*Stenella coeruleoalba*
Laminaria rodriguezii			*Steno bredanensis*
			Tursiops truncatus
			Ziphius cavirostris
	Echinodermata		
	Asterina pancerii		
	Centrostephanus longispinus		
Rhodophyta	*Ophidiaster ophidianus*		
Goniolithon byssoides		**Reptiles**	
Lithophyllum lichenoides		*Caretta caretta*	
Ptilophora mediterranea		*Chelonia mydas*	
Schimmelmannia		*Dermochelys coriacea*	
schoubsboei		*Eretmochelys imbricata*	
		Lepidochelys kempii	
		Trionyx triunguis	
	Mollusca	***Birds***	
	Charonia lampas lampas	*Pandion haliateus*	
	Charonia tritonis variegata	*Calonectris diomedea*	
	Dendropoma petraeum	*Falco eleonorae*	
	Erosaria spurca	*Hydrobates pelagicus*	
Cnidaria	*Gibbula nivosa*	*Larus audouinii*	
Astroides calycularis	*Lithophaga lithophaga*	*Numenius tenuirostris*	
Errina aspera	*Luria lurida*	*Phalacrocorax aristotelis*	
Gerardia savaglia	*Mitra zonata*	*Phalacrocorax pygmaeus*	
	Patella ferruginea	*Pelecanus onocrotalus*	
	Patella nigra	*Pelecanus crispus*	
	Pholas dactylus	*Phoenicopterus ruber*	
Bryozoa	*Pinna nobilis*	*Puffinus yelkouan*	
Hornera lichenoides	*Pinna rudis*		
	Ranella olearia		
	Schilderia achatidea		
Crustacea	*Tonna galea*		
Ocypode cursor	*Zonaria pyrum*		
Pachylasma giganteum			

Table 14. List of Species whose exploitation is regulated (***ANNEX III*** of the Protocol concerning Specially Protected areas and Biological Diversity in the Mediterranean Sea adopted in the Barcelona Convention in 1996)**.**

Porifera *Hippospongia communis* *Spongia agaricina* *Spongia officinalis* *Spongia zimocca*	**Cnidaria** *Antipathes sp. plur.* *Corallium rubrum*	**Pisces** *Alosa alosa* *Alosa fallax* *Anguilla anguilla* *Epinephelus marginatus* *Isurus oxyrinchus* *Lamna nasus* *Lampetra fluviatilis* *Petromyzon marinus* *Prionace glauca* *Raja alba* *Scianea umbra* *Squatina squatina* *Thunnus thynnus* *Umbrina cirrosa* *Xiphias gladius*
Echinodermata *Paracentrotus lividus*	**Crustacea** *Homarus gammarus* *Maja squinado* *Palinurus elephas* *Scyllarides latus* *Scyllarus pigmaeus* *Scyllarus arctus*	

Number of exotic species

Definition: Number of extra-Mediterranean faunal and floral marine species that have been unintentionally introduced or invaded and established reproducing populations and/or imported species that are subsequently living in the wild.

Rationale: Polluted or physically-degraded environments are prone to invasion more than pristine sites. A recent study of macrofouling organisms discovered that many more species were found in a polluted than in a nonpolluted marina, and that the cosmopolitan serpulid worm *Hydroides elegans* that comprised 65% of the population in the polluted marina was only infrequently found in the nonpolluted marina (Kocak et al., 1999). The mariculture introductions are mostly restricted to lagoonar or estuarine habitats, and the vessel-transported exotics to polluted harbours (Zibrowius, 1992) - environments known for their low biodiversity.

The invasion of the Mediterranean by exotic species has been mainly studied with regard to nektonic and macrofaunal organisms. Effective documentation of an invasion can be based on reliable early records of presence of faunal groups in the Mediterranean prior to the early/middle 19th century, in comparison with groups which are only recorded later than that, and therefore are attributable to transport via modern shipping, escape of captured species, or the opening of the Suez Canal. It is far more difficult to document the invasion of exotic meiofaunal elements into the Mediterranean Sea, as early records are significantly more scarce. However, benthic foraminifera have good preservation potential and may be present in large numbers, tending to leave behind a superior record of their presence over time, in comparison with macrofaunal elements.

A recent, extensive study on benthic foraminifera from the shallow continental shelf along the SE Mediterranean (Hyams, 2001) indicates that nearly 20% of the local foraminifera species are suspected to be of an exotic origin. Our ability to make this estimation may in part be attributed to recent publication of the Atlas of Recent Foraminiferida of the Gulf of Aqaba (Hottinger et al., 1993) and modern compilations of Mediterranean species (Cimerman and Langer, 1993, Yanko et al., 1998), which enable comparison of the benthic foraminifera assemblages in both regions. Moreover, their response to pollution monitoring makes them good candidates for assessing EcoQs.

Advantages: There is public and scientific awareness worldwide and exotic species are easily distinguished.

The CIESM atlases series on main exotic taxa, accessible through the internet, provide the means to distinguish among similar species

Limitations: The CIESM atlases do not cover all groups

Many areas such as N African coasts are literally unexplored.

Occurrence of nuisance species (HABS)

Definition: Occurrence of nuisance phytoplankton species of public interest. Measured as records of incidences and trends in these records in marine farms and ports (transferred in ballast tanks).

Among the many phytoplankton species existing all over the world, some are harmful species or even toxic the so called nuisance species. The most damaging harmful species are those producing toxins directly toxic to marine fauna and flora, or toxins, which accumulate in shellfish, fish, etc. The latter may subsequently be transmitted to humans through consumption of contaminated seafood and become a serious health threat. Thus, toxins are searched in shellfish etc with the aim to protect consumers. Five human syndromes are presently recognised to be caused by consumption of contaminated seafood. These are:

- ✓ amnesic shellfish poisoning (ASP);
- ✓ diarrhoeic shellfish poisoning (DSP);
- ✓ paralytic shellfish poisoning (PSP);
- ✓ neurotoxic shellfish poisoning (NSP);
- ✓ cyanobacterial toxic poisoning (Nodularin).

Harmful Algal Blooms (HABs) occur in many Mediterranean areas and may have increased in frequency with increased nutrient inputs from land. Public interest focuses mostly on the first three syndromes. A European initiative (BIOHAB project, funded by EU) aims to determine the interplay between anthropogenic and biological control of the losses and gains to HAB populations.

Advantages: The marine farms are monitored for nuisance algae. There are European networks and public interest to investigate Harmful Algal Blooms (HABs).

Limitations: Quality assessment in species determination and quantification is a problem
Differences in monitoring programmes

Dominance index

Definition:

McNaughton's Dominance Index (McNaughton, 1967): percentage of abundance contributed by the two most abundant species. The most commonly used dominance index applied in phytoplankton studies. Can be calculated for a group i.e. diatoms or for the whole phytoplankton community.

$$\delta = 100 * \frac{(N1 + N2)}{N}$$

N1, N2= the number of individuals in the two most abundant species
N = the number of individuals in a population or community

The Berger-Parker dominance index = N1/N (Berger and Parker, 1970): percentage of abundance contributed by the most abundant species.
N1 = the number of individuals in the most abundant species
N = the number of individuals in a population or community

<u>Advantages</u>: It can be easily seen that a dominance index can be calculated much easier than a diversity index, since it requires less work (identification only of the two most abundant species, therefore less problems regarding quality assurance in species identification, especially regarding rare species, etc. Furthermore, what one needs to do regarding phytoplankton is to measure a number of fields in the cuvette containing the sample under the inverted microscope and in the same fields count the two most abundant species, without having to identify them fully.

<u>Limitations</u>: Tsirtsis and Karydis (1998) evaluated dominance indices, using data from two sampling sites, one eutrophic and one oligotrophic (known *a priori* as such) as reference data. McNaughton's dominance index proved to be most sensitive for discriminating between eutrophic and oligotrophic conditions than the Berger-Parker dominance index. However, it was not sensitive enough to resolve the differences in community structure imposed by the different trophic levels in non eutrophic areas.

Presence and abundance of marine macro-phytobenthos (macrophytes)

<u>Definition</u>: Marine macro-phytobenthos includes two fundamentally different groups of plants, the seaweeds (macroscopic non vascular plants or "algae") and the seagrasses (vascular plants). Apart from their intrinsic floral values as a diverse suite of species, macro-phytobenthos communities have important ecological role in the ecosystem of the continental shelf (i.e. important primary producers, habitat builders). Especially seagrass beds form the structural base for some of the most productive coastal ecosystems of the world, including rocky and soft bottom intertidal and subtital zones, coral reefs and lagoons.

An ecological index based on macrophytes could combine the presence-absence of key species and an estimate of their abundance on hard and soft substrata. The abundance is usually expressed as surface (in hectares) covered by the macrophytes. A linear approach could also used (km of coastline), especially for seaweeds which are developed on hard substrata as a narrow belt.

<u>Rationale</u>: Phytobenthos is mentioned in the WFD as a "quality element" for the classification of marine coastal and transitional areas. Because benthic macrophytes are sessile and usually perenial organisms, they are continuously subject to stress and disturbances that are associated with changes in water quality along the land/sea interface. To these they respond directly and thus represent sensitive indicators of changes. Macrophytes are generally sensitive to water quality –particularly to turbidity, eutrophication, some chemical residues, but also trawling fisheries and exotic species competition). Thus, several marine macrophytic species have been broadly used as a successful phytobenthic indicator, to indicate shifts in the aquatic ecosystem from the pristine state to the degraded state.

The most typical example is that of *Posidonia oceanica*, which however is included in Key species and its population in the Mediterranean is monitored as "*Populations of Key species including protected ones*". The phycology group of the University of Ghent (Belgium) who has been studying seaweeds as indicators of marine benthic ecosystems not only in Belgium but in Papua New Guinea and E, SE African coast consider seagrasses as one of the best potential indicators of changes in coastal areas (Schils et al., 2001). Phytobenthos as a biological indicator of environmental quality has been used in Mediterranean lagoons (Fernandez et al., 2001: Corsica; Sfiso and Ghetti, 1998: Venice lagoon; Chryssovergis and

Panayotidis, 1995: N. Evvoikos Gulf; Panayotidis et al., 1999: Lesvos Island). In these studies it has been shown that macrophyte communities in terms of presence-absence, repartition, abundance (coverage), have specific temporal and spatial variability patterns reflecting the environmental conditions and therefore long term monitoring is necessary to make a diagnosis of the ecological status (distinguish ecologically meaningful trends). Another possibility to overcome the taxonomic complexity is to study communities form a functional point of view (groups of functionally similar species). Seaweeds and seagrasses comprise two evolutionary and physiologically different groups (Hemminga and Duarte, 2000) but have often been examined together because of morphological-functional similarities and the apparent overlap in habitats.

At a functional level, communities appear to be much more temporally stable and predictable than when examined at the species level. For example, anthropogenic stress shifts the community structure towards dominance of opportunistic species. For example, a reliable signal of increasing eutrophication is the replacement of late successional, perennial seaweeds, like *Cystoseira* spp. and *Fucus* spp. by opportunistic species like *Ulva* spp. and *Enteromorpha* spp (Harlin, 1995; Schramm, 1999).

A model to assess the ecological status of transitional and coastal waters has been recently developed by Orfanidis et al. (in press) based on the coverage of the macrophytes as a function of their functional morphology. According to it, the evaluation of ecological status is divided into five categories (from high to bad), as derived from a cross comparison in a matrix of the Ecological State Group and a numerical scoring system.

Advantages: Widely applicable (at European scale). Identification could be limited to the functional groups

Limitations: Difficult taxonomy (if identification has to reach the specific level). Different sampling methodology in the case of hard and soft bottom. Different approaches for the estimation of the abundance (eg surface for the seagrasses, linear for the seaweeds)

Table 16: Functional characteristics and growth strategies of marine benthic macrophytes (after Orfanidis et al., in press).

Longevity (Succession)	***Growth Strategies sensu Grime 2001***	***Sampled Genera***	***Ecological State Group***
Annuals (Opportunistic)	Ruderal	*Ulva, Enteromorpha, Scytosiphon* (erect phase), *Dictyota*	II
Annuals (Opportunistic)	Ruderal	*Cyanophyceae, Chaetomorpha, Cladophora, Polysiphonia, Ceramium, Spyridia*	II
Annuals (Mid-successional)	Stress-tolerant-Ruderal or Stress-tolerant-Competitors	*Acanthophora, Caulerpa, Chordaria, Gracilaria, Laurencia, Liagora*	II
Perennials (Late-successional)	Competitors	*Cystoseira, Chondrus, Fucus, Laminaria, Padina, Sargassum, Udotea*	I
Perennials (Late-successional)	Competitors	*Amphiroa, Corallina, Galaxaura, Halimeda, Jania*	I
Perennials (Late-successional)	Competitors	*Hydrolithon, Lithothamnion, Peyssonnelia, Porolithon*	I
Perennials (Pioneers to late-successional)	Stress-tolerant	*Cymodocea, Posidonia, Ruppia*	I

Presence of sensitive zoobenthic species/taxa

Definition: The presence of indicator species/genera/taxa mentioned as fragile, and slowly reproducing. The presence can be expressed eiher as absolute density per m2 or as relative abundance in percentage. This parameter/indicator is also used to calculate other community indices and highlight changes in species diversity.

Rationale : Borja et al. (2000) based on the sensitivity of zoobenthic species to an increasing stress gradient have grouped them into five clusters. The first cluster includes species very sensitive which are present only under unpolluted conditions (Group 1: specialist carnivores and some deposit feeding tubicolous polychates). Simboura and Zenetos (in press) have compiled a preliminary list of sensitive taxa according to community type.

At a higher taxonomic level it has been established that some taxa such as echinoderms and amphipods are sensitive to environmental stress. An inverse relationship was found between the richness of the Amphipod population and the degree of pollution in hard substrata (Bellan-Santini, 1980). Three groups of species have been defined (Table 17) corresponding to three classes of ecological status from very pure to polluted. It has also been documented that in sandy areas the absence or presence and relative dominance of amphipods are elements related to the environmental quality (Bakalem, 2001a). In this line the amphipod fauna was studied in three areas under different pressure in Algeria, namely: Bay of Alger: industrial and urban effluents, Bay of Bou Ismail: urban effluent, and Gulf of Jijel: pristine area. Results showed that amphipods were absent from the polluted zones of the first two areas and that the overall diversity was maximum in the pristine area (20,98% of the total diversity), lower in the Bay of Bou Ismail (17,81% of the total diversity) and even less in the Bay of Alger (9-14% of the total diversity). A list of the species present in each area is presented in Table 18.

Because densities of sensitive (vulnerable) species are community dependent, reference levels will vary among indicator species and community. The mean densities could however be regarded as lower estimates of the reference levels. Reference levels to be established from pristine communities (human pressure minimum to non-existing).

Table 17: Species groups according to ecological quality in hard substata (from Bellan-Santini, 1980)

Pure to very pure	intermediate	More or less polluted
Hyale	*Amphithoe ramondi*	*Caprella acutifrons*
Elasmopus pocillimanus	*Stenothoe tergestina*	*Podocerus variegatus*
Caprella liparotensis		*Jassa falcata*

Table 18. Amphipod species present in fine sands under different environmental stress (after Bakalem, 2001) (r): rare Underlined the dominant species.

Fine sands - Reference site	**Urban pollution**	**Urban+industrial pollution**
Ampelisca brevicornis	*Urothoe poseidonis*	*Pariambus typicus f. armata*
Ampelisca spinipes	*Urothoe brevicornis*	*Atylus swammerdami*
Ampelisca sarsi	*Urothoe grimaldii*	*Ampelisca spinipes*
Ampelisca diadema	*Ampelisca brevicornis*	*Ampelisca sarsi*
Lembos spiniventris	*Lembos spiniventris (r)*	
Urothoe poseidonis	*Ampelisca diadema (r)*	
Urothoe brevicornis	*Ampelisca sarsi (r)*	

	Siphonoecetes dellavallei (r)	
	Lembos angularis (r)	
	Phtisica marina (r)	

Advantages:

The presence of sensitive taxa is a reliable measure of ecosystem health.

Suggestions for sensitive indicator species on phyto and zoobenthic communities are given in Orfanidis et al. (in press) and Simboura and Zenetos (in press).

The EUNIS biotope classification programme promises a valuable basis for evaluation of indicators according to community type (Connor, 2000).

Limitations: The presence of sensitive taxonomic units (species/genera/taxa) is community dependent. Therefore absence from a community type does not necessarily imply disturbance. A table with sensitive taxa characterizing different communities with reference levels needs to be constructed.

Abundance of opportunistic benthic species /taxa

Definition: The density of small, short-lived, opportunistic species. The presence can be expressed eiher as absolute density per m2 or as relative abundance in percentage. Used also for calculation of community diversity (H) and other biotic indices.

Rationale: Impact studies worldwide have shown that a great number of phyto-, zoo benthic species is indifferent to human/environmental pressure. These species, the so called opportunists, tend to dominate at the expense of other sensitive species which are eliminated. These opportunistic species can be regarded as suitable indicator species for disturbance. Benthic species/taxa indicative of environmental disturbance have been reviewed by Rygg (1995), Borja et al (2000) and in the Mediterranean by Orfanidis et al. (in press)[phytobenthos] and Simboura and Zenetos (in press) [zoobenthos] who have compiled preliminary lists. These species may be present in more than one community types. However, species density is different among community types and reference levels and EcoQOs should be formulated for every species for each community type separately. The mean densities could be seen as highest estimates of the reference levels. Reference levels are formulated for minimal human pressure. Reducing human pressure should therefore decrease the densities of these opportunistic species. Polychaetes: Polychaete species belonging to families like Spionididae, Capitellidae and Cirratulidae are considered good candidates for this indicator. The usage of polychaetes in assessing impact from river inputs and river discharges has been demonstrated by many workers and recently by Cardell et al. (1999).

Nematodes: The utility of the nematode component of the meiofauna as a tool for assessing disturbance has been demonstrated with various stressors. Changes of nematode abundance in response to dredged material disposal at a variety of locations have been documented around the U.K. coast (Boyd et al., 2000).

Based on a synthesis of reviews on the subject and on data gathered from Mediterranean areas (Dauvin, 1993; Pearson and Rosenberg, 1978; Bellan, 1985) the following table (Table 19) shows the zones of pollution with the respective key macrozoobenthic species in sandy-muddy communities.

Table 19. Species, indicative of the degree of environmental status (after Zenetos and Simboura, 2001)

1	Zone of maximal pollution	Azoic
2	Highly polluted zone	Opportunists*: Capitella capitata*, *Malacoceros fuliginosus Corbula gibba*
3	Moderate polluted zone	Opportunists: *Chaetozone sp, Polydora flava, Schistomeringos rudolphii, Polydora antennata, Cirriformia tentaculata*
4	Transitional zone	Tolerant species: *Paralacydonia paradoxa, Protodorvillea kefersteini Protodorvillea kefersteini, Lumbrineris latreilli, Nematonereis unicornis*, *Thyasira flexuosa*
5	Normal zone	Sensitive species ex. Syllis sp.

Advantages:

Suggestions for potential indicator species on phyto and zoobenthic communities are given in Orfanidis et al., (in press) and Simboura and Zenetos, 2002.

-The EUNIS biotope classification programme promises a valuable basis for evaluation of indicators according to community type (Connor, 2000).

Limitations:

- indicator species are community dependent.

-Reference levels and scales for EcoQs should be established for these indicator species for each benthic community.

Community diversity (H)

Definition: Diversity as calculated using the Shannon-Wiener formula (*H*) (Shannon and Weaver 1963):

$$H = -\sum_{i}^{s} P_i \log_2 P_i$$

where $P_i = n_i / N$ (n_i the number of individuals of the *i*th species and *N* the total number of individuals) and *s* the total number of species. High diversity values normally are correlated with high numbers of species and indicate beneficial environmental conditions.

The number of species and their relative abundance can be combined into an index that shows a closer relation to other properties of the community and environment than would number of species alone. The Shannon-Wiener diversity index, developed from the information theory, has been widely used and tested in various environments. Although it reflects changes in the dominance pattern, it has been argued that it is no more sensitive than the total abundance and biomass patterns in detecting the effect of pollution and is more time-consuming.

When evaluating H, one should take into account separately its two components together with the faunistic data, in order to detect extreme abundance of opportunists indicating disturbance. There are some cases where the diversity is significantly high, even higher than normal, whereas the community is disturbed. The ecotone point is a transitional zone between two succesional stages after which the community returns to normal. The community at the ecotone point consists of species from both adjacent environments (enriched and less enriched). After the ecotone point the community often reaches a maximum in the number of species, probably due to the presence of bith sensitive species recolonising community and tolerant species, while abundance declines to a steady state level usually found in normal communities. Thus diversity may become higher than that of the normal communities (Pearson and Rosenber, 1978; Bellan, 1985).

The values of community diversity are influenced by sample size, sampling methodology and identification procedures. Consequently, species diversity values can only be compared if the same sampling methodology has been followed, with equal efforts of taxonomic scrutiny. Only under these conditions of quality assurance, trends or changes in species diversity can be investigated, such as has been done for experimental plots by Lavaleye (2000) for the North Sea and Simboura and Zenetos (in press) in the Mediterranean Sea.

Community diversity in Greek waters ranges between 1,82 to 6,68, if calculated on pooled data. However, if calculated on a standard sampling unit ($0.1m^2$) the maximal value is 6,06 bits/unit. Table 20 presents the range of diversity values estimated per community type

Table 20: Range of Community diversity (H) according to sampler (0.05. 0.1, 0.2, $0.5m^2$) and community type (after Simboura and Zenetos, in press).

Community type	**H min (disturbed to polluted)**	**H max (undisturbed)**
Midlittoral sands	0.57-1.31 (Thermaikos)	1.12-1.40 (Strymonikos)
Deltas	$0.85/0.2m^2$ (Evros)	$3.74/0.2m^2$ (Strymonikos)
Lagoons	$0.78/0.1m^2$ (Logarou)	$3.29/0.1m^2$ (Papas)
Muddy sands	$3.5/0.1m^2$ (Saronikos, Izmir)	$5.67/0.1m^2$ (Petalioi)
Muddy sands with phytal cover	$3.5/0.1m^2$ (Turkey)	$5.21/0.1m^2$ (Ionian)
Sandy muds	$1.99/0.1m^2$ (Saronikos)	$4.94/0.1m^2$ (Pagassitikos
Shallow muds	$3.17/0.1m^2$ (Maliakos)	$4.97/0.1m^2$ (Strymonikos)
Deeper muds	$2.36/0.1m^2$ (N Evvoikos)	$4.04/0.1m^2$ (S. Evvoikos)
Shallow Sands	$1.82/0.5m^2$ (Marseille)	5.16/0.5 (Milos isl./Kyclades)
Deeper Sands with detritus	$2.87/0.1m^2$ (Ionian)	$5.22/0.1m^2$ (Ionian)
Deeper Coarse sands	$3.74/0.1m^2$ (Ionian)	6.06 $/0.1m^2$ (Strymonikos)
Shallow muddy sands	$2.35/0.05m^2$ (Geras)	$5.23/0.05m^2$ (Oropos)
Coralligenous	$4.84/0,1m^2$ (Chalkis)	$5.16/0.1m^2$ (Ionian)

Kabuta and Duijts (2000) reported an increase in the diversity of macro zoobenthos (Shannon Wiener Index value) in Dutch coastal waters in the period 1995-1998, which was attributed to a decrease of dominance. They agreed that H may be used as an indicator of ecological quality. Similarly, Anonymous (2000) have used H as a tool and have further advanced into classifying the ecological quality status of their waters according to the scale seen in Table 21. An effort to classify Greek Waters with a similar approach was attempted by Zenetos and Simboura (2001).

Table 21 Classification diversity (H) of soft-bottom fauna (EEA, 2001)

Parameters	Classes				
	I **Very Good**	**II** **Good**	**III** **Fair**	**IV** **poor**	**V** **Bad**
Shannon-Wiener index (H) (Norway)	**>4**	**4-3**	**3-2**	**2-1**	**<1**

Phytobenthos

The Shannon Wiener diversity index based on benthic vegetation has been successfully used as ecological quality descriptor in the eastern Mediterranean (Chryssovergis and Panayotidis, 1995; Panayotidis and Chryssovergis, 1998; Panayotidis et al., 1999; Monterosato and Panayotidis, 2001).

Advantages: Although it is influenced by size of sampling it is less so than species variety.

Limitations:

Need for detailed identification down to species level (high taxonomic competence).
Moderately dependant on community type.

Not applicable for colonial organisms that make-up the majority of the species on hard substrata.

When evaluating H, one should take into account its two components (H, J), together with the faunal data in order to detect extreme abundance of opportunists indicating disturbance. For instance, in transition zones between two succession stages, diversity may be high, even higher than normal, whereas the community is disturbed.

Biotic index(BC)

This is a Biotic Coeficient designed (Simboura and Zenetos, in press) to classify benthic communities to an Ecological Quality Status (EQS) according to the WFD requirements (EEC, 2000). It is based on the initial idea by Borja et al. (2000) and modified to become more simple in its use.

The zoobenthic species are classified into three ecological groups and assigned a score from 1 to 3 according to their degree of tolerance or sensitivity towards pollution. The three ecological groups are:

Group 1 (GI). Species with score 1 are sensitive to disturbance in general. Species indifferent to disturbance, always present in low densities with non-significant variations with time are also included in this group. This group corresponds to the k-strategy species, with relatively long life, slow growth and high biomass (Gray, 1979).

Group 2 (GII). This group includes species with score 2. Species tolerant to disturbance or stress, whose populations may respond to enrichment or other source of pollution by an increase of densities (slightly unbalanced situations). This group includes also 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.

Group 3 (GIII). Species with score 3. First order opportunistic species (strongly unbalanced situations), pioneers, colonisers, species tolerant to hypoxia.

Following many calculations, validation and testing with data from Greek ecosystems as well as Mediterranean, an algorithm is developed giving different weight to the presence/abundance of each group:

Biotic Coefficient = { 6 X %GI) + 2 X (% GII + % GIII)}/100

The BC takes continuous values from 2 to 6, and equals 0 when the area/community is azoic. A classification system (Table 22) appears as a function of BC including five levels of ecological quality status (EQS).

Table 22. Classification of EcoQ according to range of the Biotic Index (source: Simboura and Zenetos, in press).

Pollution Classification	**BC**	**Ecological Quality Status (EQS)**
Normal/Pristine	$4,5 \leq BC < 6$	High
Slightly polluted, transitional	$3,5 \leq BC < 4,5$	Good
Moderately polluted	$2,5 \leq BC < 3,5$	Moderate
Heavily polluted	$2 \leq BC < 2,5$	Poor
Azoic	Azoic	Bad

Advantages: The BC proposed is simple in its use, it is not community type specific or site specific (global application), it is robust (not affected by sample size) and effective in its use as it encompassed both the traits of community structure (evenness of distribution) and species composition according to the species' ecological properties.

Limitations: Limitations of the use of the BC are met in the case of transitional waters where the natural conditions favour the presence of tolerant species in very high densities. In this case undisturbed lagoons may appear with low quality status if the biotic coefficient is used. Other indices such as the geometric body size distribution may be more reliable (Reizopoulou et al., 1996).

Another case where the BC should be treated with caution taking into account the natural conditions and limitations of the ecosystem is in the case of enclosed muddy bays. In this case the nature of the substrate, with high percentage of fine particles, favours the accumulation of organic matter. Thus, the benthic fauna is normally dominated by some tolerant, mud loving species (scored with 2), a fact which may lower the BC to the scale of good quality status and not the highest quality, even if the conditions are undisturbed by human activities. In other words these ecosystems may be considered as naturally "stressed" and in these cases the second scale of quality labeled as good should be considered as "very good" as this actual grade of scale may be missing.

Another important limitation is that the life strategy of all the species (or most at least) has to be known.

Additional indicators

Pielou's index of Evenness

Definition:The eveness of distribution J according to Pielou (1969) is expressed as

$$J = \frac{H'}{H'max}$$

where $H'max = \log_2 S$ S = the total number of species

This index is a measure of the distribution of individuals among species. Low evenness values indicate an uneven distribution with high densities of only few opportunistic species.

Advantages: does not depend on sample size.

Limitations: not very effective and robust in pollution assessments

Comparison of dominance curves

The dominance curve is a technique for graphical representation of species abundance (or biomass) patterns in a sample in which species are ranked by abundance and the percentage of the total number of individuals belonging to each species is plotted against (log) species rank (Clarke, 1990). These percentages are cumulated in the "k-dominance curves" of Lambshead et al., 1983 or separate k-dominance curves for abundance and biomass are superimposed giving the "**Abundance-Biomass-Comparison" ABC curves** of Warwick (1986). The degree and direction of separation of the ABC curves is expressed by Clarke's W-statistic (Clarke, 1990; Clarke and Warwick, 1994).
The W-statistics is calculated from the percentage difference in cumulative biomass (B) and cumulative abundance (A) of the species *i*:

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

This index is scaled so that complete biomass dominance and an even abundance distribution gives a value of +1 and the reverse case a value of _1.

The Abundance-Biomass Comparison (ABC-method), which combines the size, biomass and relative abundance of the species in the community has been extensively and successfully used in many incidents of acute or chronic anthropogenic disturbance with benthos data. ABC comparison curves and W-statistics have been tested in Mediterranean lagoons (Greece) (Reizopoulou et al., 1996).

The k-dominance curves have been tested in Elefsis Bay (Aegean Sea) and a N. Evvoikos (Aegean Sea) dumping site for assessing pollution impact on benthic communities

(Nicolaidou et al., 1993) and the ABC comparison curves in Saronikos gulf, with success (Simboura et al., 1995).

Advantages: a) seems particularly useful in the situation where polychaetes increase after disturbance. b) The method combines data of all benthic species in one analysis, without loosing the community perspective. c) The body-size distribution among the species in the community is related to the functioning of the community as a whole (Peters and Wassenberg 1983), and can be regarded as an indicator for the Production/Biomass ratio of the community. d) It combines abundance and biomass data per species in one analysis. The methodology has the best link with the theoretical concept of community structure (May 1984).

Limitations: a) It does not necessarily describe changes in benthic community structure generated by human disturbance alone, as they are also sensitive to natural perturbations.
b) the ABC curve demands detailed taxonomic work as the community diversity index (Shannon-Wiener) c) contrasting patterns have been found for different communities (Beukema 1988, Damuth 1991), which makes it difficult to apply them for reference levels or EcoQOs (combination of the ABC-method and Partial Dominance Curves proposed) d) Overdependence on the single most dominant species (Clarke, 1990)

Log-normal distribution

The distribution of individuals among species departs from the log-normal model of distribution (or many geometric classes are covered) when data from polluted areas are plotted, while the distribution fits the log-normal model when data from unpolluted areas are plotted (Gray, 1980).

Limitations: a) doubts have been expressed as to whether undisturbed benthic communities conform to this model empirically or on theoretical grounds (Warwick, 1986). b) Departure trends from the log-normal distribution should be treated cautiously as pollution is not the only factor leading to a lack-of fit to a log-normal (Gray, 1983). c) Insufficient or inferior to diversity index in detecting community changes induced by heavy-metal pollution (Rygg, 1986).

Geometric abundance/size classes distribution

In this technique the percentage of species is plotted against the number of individuals per species in geometric abundance classes (Gray and Mirza, 1979; Gray and Pearson, 1982).
The method of geometric abundance classes distribution has not been proved very effective in lagoons whereas the geometric size (biomass) classes distribution was very effective and sensitive in discriminating between different disturbance levels in lagoons (Reizopoulou et al., 1996).

The ratio between r- and K- selected species

Definition: The ratio of the smaller r-selected or opportunistic species versus the competitive dominants traditionally regarded as k-selected or conservative species. This ratio calculated by the Abundance-Biomass Comparison (ABC) methodology and the derived W-statistics is used to indicate changes in community structure (De Boer et al., 2001).

Advantages: a) it combines data of all benthic species in one analysis, without loosing the community perspective; b) the body-size distribution among the species in the community is related to the functioning of the community as a whole (Peters and Wassenberg 1983), and can be regarded as an indicator for the Production/Biomass ratio of the community or the energy use and transfer within the community (Cyr *et al.* 1997aandb).

Limitations: a) it does not necessarily describe changes in benthic community structure generated by human disturbance alone, as they are also sensitive to natural perturbations.
b) by calculating only the r/K ratio, nothing is known regarding the underlying cause for the shift in the ratio, whereas changes in the rank-abundance graphs can be attributed to particular species; c) it is difficult to impossible to know the exact score of a certain species on the r/K scale.

Several approaches have been applied to get around this last limitation. These are summarised by De Boer et al. (2001):

The approach used by Frid *et al.* (2000) was to describe the biological traits of each species: size, longevity, reproduction type, adult mortality, attachment, adult habit (sessile, swimming, crawling, burrowing, or in crevice-dwelling), body flexibility, body form and feeding habit. The biological trait analysis is hampered by the availability of sufficient knowledge of all benthic species.

Another approach was tried in the GONZ III project (Holtmann 1999) in which a rough cut-off point was used to discriminate between r- and K-selected species. By using size and weight as criteria r-species (maximum size <3 cm, maximum weight <0.5 mg AFDW) were distinguished from K-species (>3 cm, >250 mg AFDW).

By constructing biomass size-spectra for benthic communities undisturbed communities can be distinguished from disturbed communities, such as has been done for a Spanish estuary in Saiz-Salinas and González-Oreja (2000). This approach is relatively similar to the density-body size relationship illustrated in Cyr *et al.* (1997ab).

Infaunal trophic index(ITI)

Functional groups The macro zoobenthos species can be divided into: (1) suspension feeders; (2) interface feeders; (3) surface deposit feeders; and (4) subsurface deposit feeders. Based on this division, the trophic structure of macro zoobenthos (Infaunal Trophic Index = ITI) can be determined using the formula:

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

in which n_1, n_2, n_3 and n_4 are the number of individuals sampled in each of the above mentioned groups. ITI values near 100 means that suspension feeders are dominant and that the environment is not disturbed. Near a value of 0 subsurface feeders are dominant, meaning that the environment probably is disturbed strongly due to human activities. ITI was used successfully in the North Sea (Holtmann, 1999).

Kabuta and Duijts (2000) found one area north of the Wadden Sea where the Infaunal Trophic Index value clearly decreased in the period 1991-1998.

Advantages: May be useful in determining the ecological state of coastal waters and probably it may be applied for trend analysis.

Limitations: Integrated indicators like the Infaunal Trophic Index (ITI), require a larger monitoring effort than simple, directly determined, variables. It is useful only when a background database is available pertaining to the specific ecosystem.

Changes in the distribution area of habitat types

Rationale: The more sensitive coastal habitat types in the Mediterranean are defined and partly mapped (Spain, France, Italy, Greece). This could be easily accomplished for ALL Mediterranean countries. Human derived stress will first impact these habitats. If a protocol for rapid assessment syrveys is developed and agreed upon, then based on the changes on the changes in habitat distribution of a few “Key species”, a clear sign of environmental degradation will be easily discerned and quantified.

Rapid assessment techniques (e.g. rapid ecological assessment or side-scan for landscape diversity) and in particular specific surveys of species considered as “key-species” for marine biodiversity are gaining increased attention. Among the species of a region, “key-species” are those that contribute to the architectural, trophic and functional complexity of a marine ecosystem. Those which according to the BIOMARE (10.2001 workshop) have been cited as directly related to known stressors, are tabulated in Table 23. Air borne photography for example is a fast way to define surface versus potential surface of coverage of key phanerogams or sponges.

Advantages: Fast, accurate estimation of population size. Categories of threat to individual species can be assigned. Provides a manual for biodiversity managers

Limitations: Expensive (remote sensing). Needs testing. Not applicable to all Seas in Europe.

Table 23 : species cited as "key-species" for the Mediterranean region BIOMARE (updated 10/01)

	Species	Type (rare, endemic, keystone, threatened, biogenic building, emblematic)	Known stressors
PHANEROGAMS	*Posidonia oceanica*	Keystone, patrimonial	Eutrophication, pollution, turbidity, invasive species etc.
	Ruppia maritima	Threatened	Eutrophication, pollution, turbidity, etc.
	Zostera noltii	builder	Eutrophication, pollution, turbidity, etc.
	Cymodocea nodosa	Builder, keystone	Eutrophication, pollution, turbidity, etc.
SPONGES	*Spongia spp.*	commercial, endemic, threatened	Fishing, climate change
	Asbestopluma hypogea	Endemic	global change
	Oopsacas minuta	Endemic	global change
CNIDARIANS	*Cladocora caespitosa*	builder	climate change
	Corallium rubrum	commercial, endemic	Fishing, climate change
	Eunicella spp.	keystone	Climate change
	Paramuricea clavata	keystone, endemic	Climate change, fishing, diving, shipping, anchoring
ECHINODERMS	*Centrostephanus longispinus*	threatened	Climate change
CRUSTACEA	*Scyllarides latus*	Threatened, commercial	fishing
MOLLUSCA	*Lithophaga lithophaga*	threatened	loss of habitat, fishing
	Patella ferruginea	threatened	loss of habitat, tourism
	Pinna nobilis	threatened	loss of habitat
PISCES	*Sciaena umbra*	threatened	Spearfishing
	Epinephelus marginatus	endemic, emblematic	Spearfishing
	Cethorhinus maximus	rare	
	Carcharodon cacharias	rare	
	Hippocampus spp.	threatened	loss of habitat
	Aphanius fasciatus	rare	
TURTLES	*Caretta caretta*	emblematic, threatened	Fishing, shipping, loss of habitat, pollution
MAMMALS	*Monachus monachus*	emblematic, threatened	Fishing, shipping, loss of habitat
	Tursiops truncatus	emblematic, threatened, keystone	Fishing, shipping

9. INDICATORS ACCORDING TO HUMAN ACTIVITY IN THE MEDITERRANEAN

Dumping

Dumping as defined by the Barcelona Convention protocol is any deliberate disposal or storage of wastes or other matter in the sea from ships or aircraft. The effects of dumping mineral residues (metalliferous slug, fly ash, red mud etc.) and dredged sediments on benthic communities bear great similarities and are summarized in Table 24 while the effects of sewage sludge disposal are seen on Table 25. The primary impact of dumping on the ecosystem arises from suspension and settling of particulate material resulting to turbidity and burial. Turbidity reduces the growth of algae and primary production. The mechanical burial of the benthic animals, is usually the principal effect of dumping on benthic communities, resulting to total depletion or disturbing the communities' balance (reduced diversity, species richness and evenness of distribution and/or total abundance) and is most evident in recent dumping sites. Also a major effect of sediment dumping is the persistent changes in sediment texture.

The soluble components of the discharge (ie. Dissolved or leached metals), mainly in case of mine residues, may be toxic to organisms and may influence the food chain through the process of bioaccumulation. It is noteworhty that in recent literature (Burd, 2002) metal toxicity from mine residues (Cu) has been cited as the primary factor affecting benthic communities and the mechanical disruption caused by dumping as the secondary factor.

The effects of dumping on the community level greatly depend also on the volume and sedimentological characteristics of the discharged material in relation with the local conditions, duration of dumping, water depth, surface and hydrography of the disposal area, the time of the year, the type of community in the disposal area, and the chemical composition of the discharged material. Generally deposition have greater effects on the shallower more productive zone, as deeper benthos contributes less to an area's biological productivity.

In the case of dumping of sewage sludge the impact on the benthic community is similar to that of pollution from organic enrichment.

Table 24. Effects from dumping mineral waste and dredged material

Factor (parameter of intensity of effect)	**Effects**	**Biological response**
Turbidity (amount)	decreased illumination	Reduction of algal growth and primary production (Littlepage et al., 1894)
Mechanical disruption- Burial or sedimentation, substrate instability- (amount-concentration of discharged material and rate of dumping)	Impact on the biotic communities (depletion or disturbance of balance)	Depletion to reduction of species richness Decrease of sensitive species Increase of opportunistic species or families Reduction of evenness index Reduction of diversity index Decrease of population densities, biomass (red mud flow, fly ash, copper mine tailings) Modification of feeding modes in the affected zones by red mud (Bourcier and Zibrowius, 1972; Harvey et al., 1998; Herando-Perez and Frid, 1998; Boyd et al., 2000; NCMR, 1995;1998; Vivier, 1976; Burd, 2002).

Similarity of sediments in dumped/dredged material and disposal areas	Effect on benthic communities if dumped material is coarser	Decrease of species typical of the initial community Settlement of some species preferring coarser sediments Increase of species of wide ecological requirements and eventually of sub-pollution indicator species The resulting community is degraded (reduction of evenness) and unstable though species richness may have increased (Nicolaidou et al., 1989; Salen-Picard, 1981)
	Effect on benthic communities if dumped material is finer	Reduction of total abundance, species richnness, diversity index (NCMR, 1995; Roberts et al., 1998)
Food supply in dumped material	Effect on benthic communities	Increase in density of opportunistic families (Harvey et al., 1998)
Dissolving Or leaching of metals or toxic substances (concentrations)	Trace metal contamination (incorporation) of sediment and bioaccumulation of metals by organisms affecting the food chain, toxicity repellement of drifting larvae from settling	Reduction of abundance and diversity. (Ellis and Taylor, 1988) Acute toxicity of mine tailings to marine species (Mitchell et al., 1985). Avoidance or short term reductions in fish growth in mine tailings (Johnson et al., 1988)

Table 25. Effects from dumping of sewage sludge

Factor	**Effects**	**Biological response**
Organic material load (amount) Heavy metals and industrial wastes	impact on benthic communities	Reduction of number of species, Index of diversity H Heip Index of evenness (Moore and Rodger, 1991). Low amounts of org. C with absence of toxics allows the presence of a normal community but of enhanced abundance and biomass (Eleftheriou et al., 1982).

Industrial wastes

As industrial wastes are considered waste discharges from sewage and industrial plants and include a) heavy metals b) other hazardous substances from sea-based activities (excluding oil, PAHs and anti-fouling substances) produced by the offshore oil and gas industry (e.g. benzene, phenols, benzoic acids, barium), and through shipping (e.g. phosphorous, ore, pesticides, and lipophilic substances; and c) thermo-electric and hypersaline waste water.

The effects of heavy metals (iron, nickel, lead, copper, chromium, zinc etc.) into the marine environment are considered by many to be a serious pollution problem. Heavy metals enter the marine environment a) by surface run-off from rain, b) by direct fall-out from air into the ocean and c) by waste discharges from sewage and industrial plants. Some of the main biomarkers used today in ecotoxicological research in relation to contaminants are indicated in Table 26. The lethal effects of metals on marine organisms have been extensively studied while more limited are data concerning the long-term effects on organisms (reproduction etc) and communities (see Table 27). Recent literature (Burd, 2002) has proved that sediment copper levels associate with decline in species richness and abundance of sensitive species inferring that toxicity from metals may be a major factor affecting benthic communities.

Table 26. Biomarkers and their significance

CONTAMINANT	BIOMARKER	WARNING*
Heavy metals		
Cu, Hg, Ag, Zn, Cd, Pb	DNA changes	A
	Metallothioneins	AB
	Immune response	A
	MFO	A

***** A= signal of potential problem, B= definitive indicator of type or class of pollutant

Table 27. Effectes from discharge of warm thermo-electric water and of heavy metals from sewage or industrial plants

Factor	**Effects**	**Biological response**
Warm thermoelectric water: High temperature and hydrodynamism	Impact on hard substrate superficial zoobenthos	Reduction of number of species in the warmer water area. Increase of species distribution in the high hydrodynamism area Maximal size and vitality of *Balanus* in the discharge point (Arnaud et al., 1979)
Heavy metals	Short term effects on benthic organisms	Lethal concentrations
Various	Long term effects on benthic organisms	Effect on reproduction rate and offspring survival (Reish, 1978) -in seaweeds inhibition of reproduction and development changes in community structure (Coehlo et al., 2000) in seaweeds no direct effect

Copper	Impact on benthic communities	Decline in species richness and abundance of sensitive species coinciding with high sediment copper levels (Burd, 2002)
Chromium (tannery wastes)		High temporal fluctuations of diversity and evenness indices Decrease of diversity and evenness Increase of species indicating disturbance (Papathanassiou and Zenetos, 1993)
Discharge of hyperhaline wastes		
Increase of water salinity and temperature	Impact on benthic communities	-decrease of abundance at the discharge site for all groups particularly crustaceans, echinoderms, and molluscs -reduction of species richness at the discharge point (Castriota et al., 2001) no effects on H and J at a few meters distance from the diffuser -in seagrasses species displacement e.g. *Cymodocea* instead of *Ruppia* - in seagrasses further penetration in estuarine ecosystems
(Distance from discharge point)		

Oil spills and PAHs

The impact of oil pollution on the environment in the Mediterranean is particularly severe due to the topography and hydro-meteorological conditions of the Mediterranean: oil entering or discharged there has little chance of leaving, and stays and accumulates until it is degraded. Since the United Nation's Conference on Human Environment in 1972 the protection of the Mediterranean has become a priority problem and enormous efforts have been made in organizing expert consultations and intergovernmental meetings to establish and carry out the 'Action Plan for the Mediterranean' (approved in 1976). More recent studies and Conventions now pave the way for restricted or prohibited oil discharges and better and more concentrated oil clean-up techniques (Lourd, 1977). PAH pollution may be caused by accidental oil spills, by shipping but also by industrial accidents like that of the fire in the refinery situated at Izmir Bay (Turkey, Marmara Sea) after the earthquake of 1999 (Okay et al., 2001). Oil pollution affects the ecosystem by the petroleum hydrocarbons entering the ecosystem by sea water, sediments and organisms (for details see Table 28). Oil substances may have toxic effects on organisms and may be estimated on the organismic level by measuring the PAH concentrations on tissues or through various bioassays like the **Lysosomal stability test** or the feeding rates in mussels. On the population or community level oil pollution may be lethal causing depletion of all biota at the very site of pollution. Secondary results of pollution are successional disturbance of the community structure, reccession of the sensitive species and dominance of opportunistic ones. The communities recover undergoing various stages of successional colonisation. The rate of recovery depends on the distance from the pollution source (oil spill etc), the amount of pollution load and the type of the ecosystem. For example the Gialova lagoon (southwest Greece), one of the richest Mediterranean lagoons, recovered within 2 years of an oil spill accident in the area (Dounas et al., 1998).

Table 28. Effects of oil spills and PHA on EcoQs.

Factor (parameter): Concentration of oil, type and amount of hydrocarbons, rate of degradation (distance from spill, time lapse)
Impact: -Accumulation of petroleum hydrocarbons (PAH) to sediments, interstitial water and organisms tissues -Impairement of growth and reproduction-death of biota -Physiological and behavioural abnormalities -Greatest impact on intertidal and subtidal zones -Acute or chronic toxic effects on planctonic or benthic animals -the amount of oil may gradually decrease from the sea water and increase in sediments after the accident (Gouven et al., 1996)
Recovery (extend of pollution type of ecosystem): Recovery 1->10 years. Ex. Fine sand *Abra alba* community of Bay of Morlaix >10 yrs (Dauvin, 1998) Gialova lagoon community: 2 yrs (Dounas et al., 1998)
Biological response **Organismic level: Biomarkers** DNA changes, MFO, Immune response, Lysosomal stability and Feeding rate in mussels (Okay et al., 2001). **Population level:** High mortality rates of all species (defaunation) (plants, crustaceans, fish, birds) immediately after the spill Successive colonisation by opportunistic species dominating or monopolising the fauna (1 year after) -Establishment of an unstable community (with great successional fluctuations) of low density, diversity, species richness and evenness characterised by dominant and subdominant (>10%) resistant to oil species (mainly polychaetes and gastropods). Reduced abundance of sensitive species. (Bondsdorff et al., 1990; Chasse, 1987; Sanders et al., 1980). -Total abundance may rise due to the increase of oppotunists (NCMR, 2001). -in seaweeds short-term growth reduction in intertidal species (Lobban and Harrison, 1994) -in seagrasses no direct effect -shift in planctonic species composition as a long-term trend, no significant effects (Batten et al., 1998)

Shipping: Anti-fouling substances (organotins: **TBT)**

Early in the 1970s organotins [monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), monophenyltin (MPT), diphenyltin (DPT) and triphenyltin (TPT)] were introduced as effective components in marine antifouling paints. They soon proved extremely effective for their purpose and became common world-wide. But not without causing serious ecological problems.

At the organismic level, the biological effects can be studied in relation to organic compounds through the biomarkers indicated in Table 29. In practice, serious environmental impact on non-targeted aquatic organisms was found in the form of high toxicity (Cima et al., 1996; Kannan et al., 1996; Bressa et al., 1997), reproductive performance (Franchet et al., 1999), high potential for bioaccumulation (Marin et al., 2000), and specific long-term effects known as imposex. The case of imposex in prosobranchia [Gasteropoda] following exposure to tributyltin (TBT) classify TBT among the so called endocrine disrupting chemicals (EDCs).

Restrictions for the use of tributyltin (TBT) were adopted in many countries but usually only valid for vessels less than 25 m in length. Despite restrictions on the use of organotin-based marine antifouling paints imposed throughout the Mediterranean in 1991, the concentrations encountered in 1996 in the French Mediterranean coast (Cote d'Azur) (Tolosa et al., 1996) represent an ecotoxicological risk although, compared with concentrations from previous surveys (1988), contamination from TBT is substantially less. Also along the Catalan coast, data of 1998 evidence the occurrence of organotin pollution far from the source, with levels of both TBT and triphenyltin (TPhT) high enough to cause environmental concern (Sole et al., 1998). Another study dealing with the distribution and fate of tributyltin in surface and deep waters of the northwestern Mediterranean concludes that contrary to results of coastal experiments, the half-life of TBT in this oligotrophic environment is estimated to be several years and that the ubiquity and persistence of TBT in these waters is a new source of concern for environmentalists (Michel and Averty, 1999). At the community level changes at the meiofaunal community structure were evidenced (Lampadariou et al., 1997-Aegean Sea)

Table 29. Biomarkers measured in relation to organic compounds and their meaning

CONTAMINANT	BIOMARKER	WARNING*
PCBs, DDT, HCB, TCDD	MFO	A
	Immune response	A
	DNA changes	AB
Organophosphates	Blood esterase activities	AB
Carbamates	Brain esterase activities	ABC

* A= signal of potential problem, B= definitive indicator of type or class of pollutant, C= predictive indicator of long-term adverse effect.

Fisheries

Fishing has a broad range of effects on marine and estuarine ecosystems, including: direct effects such as reductions in population sizes and shifts in the population structure of target species; effects on ecosystems caused by the removal of non-target species as bycatch; damage to habitats caused by the operational use of fishing gear; and the effects of «ghost fishing» by discarded fishing gear. In particular, the impacts of fisheries on the benthic community, have been recently reviewed by Jennings and Kaiser (1998). According to Hopkins (2000), and (ICES, 2000), the response of benthic communities depends partly on the natural spatial and temporal variability, and is highly influenced by the type of substratum. Community structure can be altered under trawling, but species richness can decrease or remain stable, due to the increased abundance of certain new, opportunistic species.

In the implementation of Ecologically Sustainable Development, fisheries management plans need to recognise effects on non-target organisms, and biodiversity generally, as potential constraints to fisheries production. The specific effects of fishing range from the impacts of non-selective bottom trawls (as used in some fish and prawn fisheries) on non-target organisms in the trawl path (Hutchings, 1990) to accidental catch of seabirds on baited hooks deployed on longlines. Some of these impacts may be substantial and important for the populations of non-target organisms.

In response to information that fisheries may be adversely affecting the environment or other species, fisheries management plans will need to adopt procedures to avoid or ameliorate these unintended effects.

Dolphins should be monitored in the Mediterranean Sea. They are very sensitive to water quality and to the quality of fish and are easy to observe.

Summarising, the effects of fishing according to literature can be discerned through the following indicators (Table 30)

Table 30. Effects of fisheries on the ecosystem biological diversity (exempting fish).

Impact on:		Suggested indicator
Bird population changes (food changes)		
Bycatch (unwanted) of mammals	Monk seal Dolphins	Trends in population
Discards	Structure of epibenthos: Large, fragile, slow-growing organisms are affected relatively more	Community diversity
zoobenthos changes		
phytobenthos	Seaweeds: damages of sublittoral stands (Blader et al., 2000) Seagrasses: fragmentation and finally decline of meadows (Sànchez-Jerez and Esplà (1996)	Phytobenthos coverage

Mariculture

Organic enrichment is the most widely encountered impact of culturing fish in cages. Therefore, the effects of mariculture on benthic communities are similar to those produced by several other sources of organic enrichment. The local decrease in diversity could be acceptable except for the following situations (Karakassis, 1998):

- The damaged ecosystem constitutes the habitat of an endangered species,
- The damaged ecosystem is a nursery ground for species affecting the ecology of a broad marine area,
- The damaged ecosystem is a rare and region-specific habitat,
- The damaged ecosystem is impaired to that extent, that its loss is irreversible on a human time scale.

The impact of cage farming of fish on the seabed has been investigated in lagoons by Lamy and Guelorget (1995) and in three Mediterranean coastal areas by Karakassis et al. (2000). The latter documented that the macrofauna community was affected up to 25m from the edge of the cages. At the coarse sediment sites, abundance and biomass under the cages were 10 times higher than at the control. However, as biomass and abundance have not been included in the hereby proposed core set of indicators, they cannot be applied to define the ecological status in mariculture sites. But the dominance of opportunistic species is a reliable indicator (Lamy and Guelorget, 1995; Karakassis et al, 2000). Indeed, *Capitella* cf. *capitata* dominated macrofauna up to 10 m from the cage in two farms, whereas the third was dominated by *Protodorvillea kefersteini*. Community diversity measured with the Shannon-Wiener index was also significantly lower in the vicinity of the fish cages and could therefore be employed as a reliable indicator. Macrobenthic community structure defined at higher taxonomic levels than species was tested against analyses at the species level. The results support the idea that monitoring data involving higher taxonomic levels (i.e. family) should be acceptable for the assessment of ecological impacts on the benthic communities (Karakassis and Hatziyanni, 2000).

Other potential indicators tested with macrobenthic data did not always proved reliable. For example the Abundane-Biomass Curves (ABC) were inconsistent with the other sources of information. In particular, even the sites close to the cages dominated by capitellids could not be classified as disturbed by the ABC curve technique. (Karakassis et al., 2000).

The scale of mariculture impacts on the ecological quality varies considerably in terms of distance and time Karakassis (1998). Consequently some could be immediately detected using the appropriate indicator whereas others cannot be traced (Table 31).

Table 31. Spatial and temporal scales of impacts related to mariculture

Impact	Spatial scale	Time scale	Indicator
Modification of gene pool of wild stocks	10-100 Km	Several generations (>10 years)	Molecular techniques/ indicators
Replacement of biota by introduced species	Depending on motility and larval propagation	Depending on life cycle	Various Species diversity indicators
Dominance of opportunists	Up to 25 m	Short-term-while lasts	Abundance of opportunistic species *Capitella spp., Protodorvillea kefersteini, Cirrophorus lyra*.
Decrease in community diversity	Up to 25 m	Short term-while lasts	-Shannon-Wiener indicator - Diversity to family level
Increase in biomass - abundance	Up to 25m	Short term-while lasts	Non reliable

Biological invasions: via shipping, with mariculture

Biological invasions in coastal ecosystems are known to have a direct impact on benthic communities. Reduced macrofauna diversity, displacement of native populations to the extinction of certain species, have been noticed in many coastal areas, especially near ports. Introduction of cultured species, and diseases through mariculture are also known in extra Mediterranean areas. Recent studies have begun to examine indirect effects of invaders and their impacts on species at different trophic levels, and impacts on food-web properties and ecosystem processes. A review of impacts by exotics at coastal areas is given by Grosholz (2002) who classifies the consequences into ecological and evolutionary ones. He presents examples of ecological consequences of invasions at various levels such as: single species impacts; multiple species impacts; multiple trophic-level impacts; ecosystem level impacts; recipient community impacts; pathogens and disease spread. Evolutionary consequences include invasion pathways; cryptic species; hybridisation with natives; plasticity in native species; population differentiation and physiological adaptation.

As derived from the above, the ecological quality of an invaded ecosystem can be defined primarily on the basis of the number of exotics present but also at the organism level (physiological adaptation) –population level (population differentiation through molecular/genetic appropriate indicator) – community level (all the proposed indicators).

10. ASSESSING ECOLOGICAL QUALITY THROUGH BIOLOGICAL INDICATORS IN THE MEDITERRANEAN

Human resources

Taxonomic accuracy sets the key to understanding history and future of marine biodiversity and moreover monitoring biodiversity functioning in different ecosystem types under different environments stress. Defining species richness in the different parts of Mediterranean may become a difficult task as discovery and description of benthic diversity in unexplored areas of the Mediterranean Sea is inevitable.

On investigating for relevant integrated works among Mediterranean countries, experts in Environmental Impact Assessment were contacted from Turkey, Lebanon, Israel, Tunisia, Spain, France and Italy. Moreover, human potential (capacity to contact relevant work) needed for EcoQOs was soughted by searching existing literature pertaining to Mediterranean areas. Below are some answers regarding current or past monitoring projects at national level.

Turkey: There is nothing on that subject along the coasts of Turkey,at the national level *(Prof. Ahmet Kideys 28.1.2002: kideys@ims.metu.edu.tr). However, there is human potential in detailed taxonomic analysis of benthic groups and sporadic studies dealing with ecological status (see Cinar et al., 1998; Cinar et al., 2001).*

Lebanon: With regard to the use of biological indicators for the definition of ecological quality status along the coast of Lebanon and the Levantine Basin, there is not a specific species to characterize ecological systems, neither at the national level. However, according to results and a long experience on the coastal and neritic ecosystems, and based on the Report on National biodiversity Study of Lebanese Marine Environment, the following are employed:

- In benthic ecosystem as indicator species among rare and threatened species the following are used/monitored: the sponge *Spongia officinalis* the mollusc *Pinctada radiata,* the appreciated peneid shrimps *Penaeus japonicus* and *P. kerathurus*.
- The meiofauna of sandy and muddy bottoms is used to assess organic and chemical pollution (group composition and the relative abundance of each group).
- The invading algue such as *Stipopodium* sp., *Caulerpa* spp. are noticed with increasing abundance overcoming some other native species.
- the sea turtles *Caretta caretta* and *Chelonia mydas* and the mammals *Monachus monachus* and *Delphinus delphis* are also subject of special survey at national level.
- Exotic species introduced in ports (Beyrouth) and other areas are investigated.

(Prof. Sami Lakkis 2.2. 2002 : slakkis@inco.com.lb)

Israel: No biological indicators are used for determination of ecological quality of coastal ecosystems off the Mediterranean coast of Israel. No bioclassification was done either. There was an attempt to map the coast by the environmental ministry, but I have not seen its results. Private research in biomonitoring work has led to accumulation of extensive data and time series of both nearshore and deep-sea benthic communities *(Bella Galil 28.1.2002:galil@post.tau.ac.il)*

Egypt: no answer

Tunisia: The studies concerning indicators among benthos are very limited in Tunisia. No national monitoring system for EcoQs with the exception of some research on *Posidonia* in some areas including some lagoon area. However, there is potential to the use of molecular techniques/genetic because of trained scientists and limited technical possibilities i.e. metallothioniins in bivalvia *(Nejla Bejaoui 29.1.2002: nejla.bejaoui@gnet.tn)*

Algeria: lot of human potential for detailed macrozoobenthos analyses. Results already available for but not sufficiently elaborated.-see Bakalem, 2001b or at a very local scale (Grimes and Gueraini, 2001a,b).

Spain: experts and experience at all levels of biological diversity evidenced by the literature. National monitoring is carried out at selected fields i.e. exotic species.

France: ibid. National monitoring is carried out at selected fields

Italy: ibid. National monitoring is carried out at selected fields

Greece: ibid. No national monitoring except MEDPOL, but many impact studies and long-term monitoring locally.

Slovenia: according to BIOMARE report, Slovenia puts a special effort in taxonomic analysis with a view to assessing the biodiversity and ecological quality of its environment.

Croatia: Research on macrophytes, exotic species and zoobenthos has started recently and is currently conducted under support by the Ministry for Science and Technology -see Ivesa et al., (2001). There is a long experience of Croatian scientists in studies of marine flora and fauna. A comprehensive potential monitoring programme of the national waters exists since 1999, which, includes biomonitoring.

Organismic level: biomarkers used in the Mediterranean Sea

Unlikely other European areas where a number of field programs has been established either at national or regional level (Conventions) and various biomarkers are applied for the measurement of the environmental condition, biomarkers in the Mediterranean have been mainly utilized through individual research projects or international programs (e.g. MED-POL) (Table 32). Within the framework of MED-POL program initial efforts have been concentrated on upgrading the technical capabilities of Mediterranean laboratories, especially those in the south. Representatives from a number of Mediterranean laboratories have participated in training courses. Moreover, individual training was organized for four techniques recommended by an expert group: lysosomal membrane stability and DNA alteration as general stress indices, and EROD and metallothionein determination as specific biomarkers (UNEP/RAMOGE, 1999). Inter-comparison exercises undertaken among the participated laboratories around Mediterranean for lysosomal membrane stability, metallothionein content, and EROD activity have produced good results (Viarengo et al., 2000). The indicator organisms used were the seabass *Dicentrarchus labrax* and the mussel *Mytilus galloprovincialis*. Yet, an EU project (i.e. BEEP) running these days where a number of Mediterranean laboratories are also participating aims to feel the gaps that are related to the use of conventional biomarkers in the different European areas and the development of new biomarkers.

Table 32. Biomarkers and indicator organisms indicated in scientific paper related to Mediterranean

A. Specimens collected from the field.

Area	Indicator Organism	Biomarker *	**Reference**
Bay of Cannes, France	*Dicentrachus labrax,* *Mytilus galloprovincialis*	EROD, GST, AChE, MTs, lysosomal stability	Stien et al., 1998a
Israel coast	*Patella coerulea, Donax trunculus, Mactra corallina,* *Monodonta turbinata*	ChE, GST, NSE, NR, DNA-unwinding assay, plasma membranes and epithelial layers permeability, phagocytic activity, MXRtr	Bresler et al., 1999
Bizerta lagoon, Tunisia	*Ruditapes decussatus, Mytilus galloprovincialis*	AChE	Dellali et al., 2001
Gulfs of Elefsis and Chalkis, Greece	*Callista chione, Venus verrucosa, Chlamys varia, Cerastoderma edule, Phallusia mammilata*	MTs	Cotou et al., 2001; Cotou et al., 1998
Gulf of Amvrakikos, Greece	*Mytilus galloprovincialis*	GPX, AChE, MTs	Tsangaris et al., 2001; Tsangaris et al., 2000
Gargour and Sidi Mansour, Tunisia	*Ruditapes decussatus*	MTs	Hamza-Chaffai et al., 1998
Israel coast	*Siganus rivulatus*	EROD, Lysosomal stability	Diamant et al., 1999
Agadir bay, Morocco	*Perna perna, Mytilus galloprovincialis*	AChE	Najiimi et al., 1997
Israel coast	*Donax trunculus*	Catalase, SOD, lipid peroxidation Imposex	Angel et al., 1999 Rilov et al., 2000
Israel coast	*Stramonita haemastoma*	Imposex	Rilov et al., 2000
Italian coast	*Donax trunculus*	Imposex	Terlizzi et al., 1998
Ebro Delta, Spain	*Procambarus clarkii*	AChE, BchE	Escartin and Porte, 1996
Catalan coast, Spain	*Bolinus brandaris*	Imposex, hormone levels	Morcillo and Porte, 1999

B. Experimentally exposed specimens to various pollutants in the laboratory.

Indicator organism	**Biomarker ***	**References**
Carcinus aestuarii	EROD, reductase enzymes, AChE, BChE, DNA strand breakage, S.L.I., S.G.I.	Fossi et al., 1996
Cyprinus carpio	VTG, P450, EROD, CYP1A, cytochromes,GST, GPX, antioxidant enzymes	Sole et al., 2000a
Dicentrachus labrax	cDNA encoding for P450 1A	Stien et al., 1998b
Aphanius iberus	HPS70	Varó et al., 2002
Ruditapes decussatus	MTs, AChE	Hamza-Chaffai et al., 1998
Ruditapes decussatus	HPS70, HPS60	Sole et al., 2000b
Procambarus clarkii	AChE, BChE	Escartin and Porte, 1996

* EROD: ethoxyresorufin-*O*-deethylase, MTs: metallothionein content, GST: glutathione S-transferase, GPX: glutathione peroxidase, AChE: acetylcholinesterase, BChE: butirylcholinesterase, ChE: chlolinesterase, NSE: non-specific esterase activity, NR: intralysosomal accumulation of neutral red, MXRtr: multidrug resistance-mediated transporter, S.L.I.: somatic hepatopangreas index, S.G.I.: somatic gill index VTG: vitellogenin, HSP70: heat stress protein 70, SOD: super-oxide dismutase.

Community level: Case Studies

Community Diversity Shannon-Wiener (H)

As mentioned in the description of the proposed indicators, community diversity ranges with community type and methodology used (sampling area, mesh size, taxonomic accuracy level). However, based on the distribution of H in 116 sites all over Greece, at a standard sampling unit (0.1m2), an arbitrary division was derived regardless of community type (Figure 6). Certainly community diversity is lowered by severe pollution stress compared with control areas or years. Values lower than 1,50 bits per unit have been calculated at the badly polluted areas of Saronikos Gulf (class I), between 1,5 and 3 for highly polluted areas of Thermaikos and Saronikos (class II), 3-4 for moderately polluted (class III) areas, 4-4,6 for transitional zones (class IV) and over 4,6 for normal zones (class V). The maximum values of H (class V) coincide with the pristine areas of Sporades marine park, Kyklades plateau, Rhodes isl., Ionian Sea and Petalioi Gulf Aegean): 6,81 bits per unit. Thus, using H, five classes of ecological status can be defined for the Greek coastal waters and a pristine area.

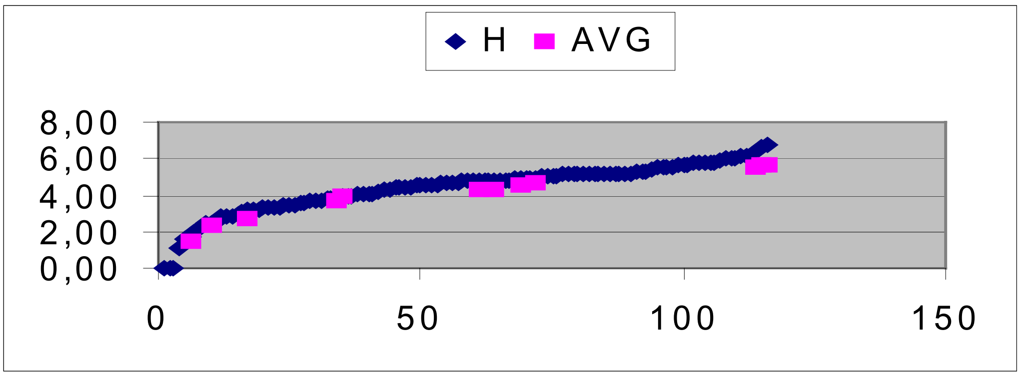

Figure 6: Distribution of community diversity (H) over 116 Greek sites. AVG: H/0.1m2 (after Zenetos and Simboura, 2001).

An even more precise division, yet somewhat arbitrary, has been suggested by Simboura and Zenetos (in press), if the variance due to community factor is eliminated. This is based on long experience of the authors in closed gulfs (Saronikos, Thermaikos): in muddy/sandy substrata. This assessment of ecological quality status according to community diversity (Box 2) is further supported by literature in other Mediterranean areas and refers to closed ecosystems, and to estimated values as means per $0.1m^2$.

Box 2: Ecological quality classes according to community diversity in closed gulfs (sandy/muddy community types).

bad:	$H\leq1,5$: Azoic to very highly polluted –examples from Elefsis Bay, Thessaloniki
poor:	$1,5<H\leq3$: highly polluted – examples from Saronikos, Thermaikos
moderate:	$3<H\leq4$: moderately polluted
good:	$4<H\leq5$: for transitional zones
high:	$H>5$: reference sites

Case study : oil spill accident

Table 33: Mid term effects of an oil spill on the macrozoobenthic species variety
Source: NCMR, 2001

Indicator of impact	**Before the oil spill (reference values)**	**1 month after**	**4 months after**	**8 months after**
S (number of species) At the site of the accident (S. Evvoikos G.) (32m depth)	23 /0,1m2	8 / 0,05 m2	16 / 0,05 m2	17 / 0,05 m2

Case study: dumping

An example of the effects of dumping on benthic communities within the Mediterranean follows below (Table 34).

Table 34:Definition and range of suggested indicators as a result of dumping. Source: NCMR, 1998

DUMPING	Area	S (number of species per surface unit	N/m^2 Density mean	H Mean	J mean
Coarse mine wastes	N. Evvoikos Gulf, Greece	30 (0,2 m2)	1797	3,76	0,78
Reference values		70 (0,2 m2)	1585	5	0,87

Case study: number of molluscan exotics

Oyster farming in Mediterranean coastal waters (Adriatic, Etang de Thau, Gulf of Gabes) is held responsible for many non-indigenous species in the area (Ribera and Boudouresque 1995). Some invaders have displaced native species locally, some are considered pests or cause nuisance, whereas other invaders are of commercial value. The tropical alga *Caulerpa taxifolia* was recorded for the first time in the western Mediterranean in 1984. It contains a toxin, which may hinder the growth of other organisms. Its rapid expansion in the basin, its distribution and ways of stopping its further spread have been the subject of many research projects, workshops and discussions. According to recent observations, it has now reached the Adriatic and threatens the eastern Mediterranean (UNEP, 1998).

The rate of marine biotic invasions has increased in recent decades; collectively they have significant ecological and economic impacts in the eastern Mediterranean.
However, at variance with other invaded seas, the invasion into the eastern Mediterranean has increased the region's biodiversity. An example is exhibited with the mollusca (Figure 7)

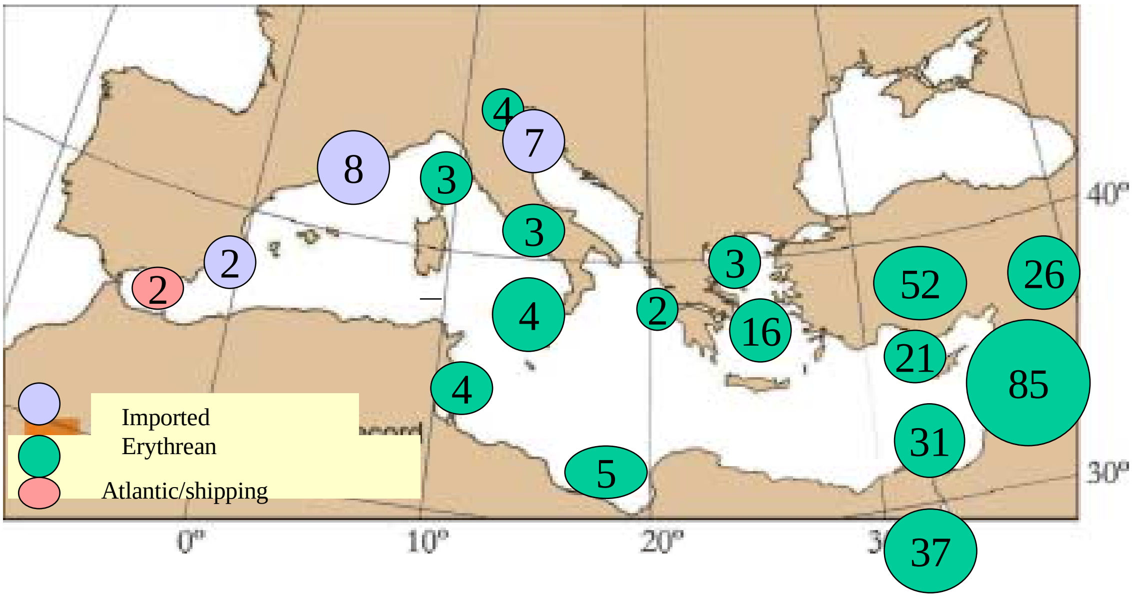

Figure 7. Distribution of exotic mollusca in the Mediterranean up to December 2000: Indo-Pacific origin (green), Atlantic (pink) and introduced via aquaculture (grey).
Source:CIESM (www.ciesm.org/atlas/)

11. CONCLUSIONS

Overall, it can be concluded that, at least at the community level, there is a lot of data collected either through impact studies or in the frame of nationally/internationally funded research projects across the Mediterranean Sea. These data have been utilized independently, incoherently and forgotten.

Of the indicators described, some (molecular, genetic, physiological) are still under the investigation stage, others are widely applied in Environmental Impact Studies, and others (involving technological development) are presently used as tools for mapping sensitive habitat types. Although they all are promising valuable tools, further work is needed before they are embedded in national and/or conventional monitoring systems.

Bearing in mind that the ultimate objective is to maintain the Ecological Quality high, that is high biological diversity in developing the ecological quality indicators, once a methodology is standardized, a scaling system should be defined for each one separately. Only then will indicators become valuable tools to managers/stakeholders, who can thus evaluate environment performance of their policies and act accordingly. For example:

<u>Indicator:</u> abundance of sensitive taxa

<u>Objective of developing it is to</u> keep high abundance and species variety of sensitive taxa.

<u>Why?</u> Because when sensitive taxa disappear it means having serious problem: disturbance to pollution.

<u>How do we measure it?</u> And how do we classify a given community/habitat/ecosystem as having bad, poor, modest, good or high quality?

The idea is correct but the ranges are missing. Which brings us back to data availability.

Data availability is indeed a primary criterion for further development of an indicator. In this sense, although human capacity is well at place in many Mediterranean countries, a lot more research is needed on molecular to organismic level indicators before methodology is standardized, results are produced, indicators are tested, and ranges are defined. Data on some populations (birds, mammals) is partly available through reports of UNEP, GFCM, EU funded projects, networks (i.e. Posidonia). However, this is not quantified to the degree required so that ranges for different ecological quality classes are defined.

On the other hand detailed data sets on benthos (phytobenthos, zoobenthos), and less on meiobenthos, community diversity and structure exist, though they are not directly accessible to the scientific society or public. However, as this data is derived on the basis of national monitoring and assessment programmes, for the moment knowledge of and experience with the use of these variables is limited. Although it is yet too early for solid conclusions on the usefulness of these variables as indicators of ecological quality at the regional level, the results of testing in different areas within the Mediterranean Sea is promising. Data holders in Research Institutes and Universities throughout the Mediterranean need to sit together and look at these data from other perspectives (validate, test them).

12. THE WAY FORWARD

There is a need of basic information. The existing data concerning biodiversity must be located. Coodination between the data holders of different data sets (phytoplankton, phytobenthos, zoobenthos) is needed so that experts reach a consensus on the best indicators and possibly ranges, primarily based with data at hand. Moreover, a trans-Mediterranean forum of experts, per research field, should decide and propose monitoring strategies per indicator, that is:

- Appropriate methodology in collecting and analyzing the data
- Appropriate spatial and temporal scales for monitoring
- Storage and assessment of data and scales of reporting

The above steps could be materialized either in the framework of existing for a i.e. the marine algae group of UNEP/MAP or through a series of dedicated workshops for this scope. The output of such workshops will be the step forward an integrated environmental approach, that is one that cares about high sustainable biological diversity.

13. DEFINITIONS

biomonitoring: The use of biological responses of selected organisms for environmental monitoring.

coastal water means surface water on the landward side of a line every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured, extending where appropriate up to the outer limit of transitional waters

community is used as a biological term in the text as synonymous to species assemblage.

dumping, as defined by the Barcelona Convention protocol, is any deliberate disposal or storage of wastes or other matter on the seabed or in the marine subsoil from ships or aircraft.

Ecological Quality (EcoQ) =**Ecological status.** As an overall expression, Ecological Quality could be expressed as a number of parameters or variables describing the physical, chemical and biological environment of a marine ecosystem. Each of these parameters or variables constitute an element of the overall expression of Ecological Quality. We will denote these as **Ecological Quality Elements**, abbreviated as **EcoQs**

Ecological Quality Objectives (EcoQOs) = **Ecological Quality Elements :** Parameters or variables describing the physical, chemical and biological environment of a marine ecosystem. EcoQO is the desired level of EcoQ relative to the reference level.

habitats (natural habitats as defined by the Council directive 92/43/EEC) means terrestrial or aquatic areas distinguished by geographic, abiotic and biotic features, whether entirely natural or semi-natural.

infralittoral zone is defined as the vertcal extent of the benthic domain which is compatible with the existence of marine phanerogams or photophilous algae (Peres, 1967).

key-species are those species that contribute to the architectural, trophic and functional complexity of a marine ecosystem. This includes taxa of great heritage value as for instance: rare, endemic, threatened, biogenic building, keystone or emblematic species.

imposex: the development of male characters in females known to occur in prosobranch gastropods.

14. ACRONYMS and web sites

BIOMARE: Implementation and networking of large scale long term MARine BIOdiversity research in Europe (http://www.biomareweb.org)
BLUE PLAN (http://www.planbleu.org)
CBD: Convention on Biological Diversity
CFP: Common Fisheries Policy
CIESM: International Commission for the Scientific Exploration of the Mediterranean Sea (http://www.ciesm.org)
DIVERSITAS (http://www.icsu.org/DIVERSITAS/)
DPSIR: (Driving forces-Pressure-State-Impact-Response) framework

EcoQOs: ecological quality objectives
EEA: European Environmental Agency
EEZ : Exclusive Economic Zone
EIA : Environmental Impact Assessment
EIONET : European Environment Information and Observation Network (http://eea.eionet.eu.int)
ERMS: European Register of Marine Species (research project) (http://www.erms.biol.soton.ac.uk)
ETC/MCE: European Topic Centre for Marine and Coastal waters (1997-2000)
ETC/WTR: European Topic Centre for Water
EuroGOOS: (www.eurogoos/org)
EUROSTAT : Statistical Office of the European Commission : (http://europa.eu.int/comm/eurostat)
FAO (the UN Food and Agriculture Organization)
HELCOM: (Helsinki Commission, Baltic Marine Environment Protection Commission)
ICCAT: International Commission on the Conservation of the Atlantic Tunas
ICES: International Council for the Exploration of the Sea (http://www.ices.dk)
ICZM: Integrated Coastal Zone Management
IRF: Inter Regional Forum (EEA)
JRC-ISPRA Joint Research Centre/Space Application Institute.
OECD: Organization for Economic Co-operation and Development : (http://www.oecd.org)
OSPAR Convention : Convention or the protection of Marine Environment of the North- East Atlantic (http://www.ospar.org) (http://www.helcom.fi/)
SDI: Sustainable Development Indicators
SDRS: Sustainable Development Reference System
WFD: Water Framework Directive
For the conservation of *Monachus monachus:* (http://www.monachus.org)
NATURA 2000, habitat types (http://www.europa.eu.int./comm/environment/nature/hab-en.htm)
Protected areas of Europe
(http://www.europa.eu.int./comm/environment/nature/spa/spa.htm)
(www.ossmed.org). The Observatory of Mediterranean Sea has put a permanent forum about "MarineProtected Areas of Mediterranean Sea" into motion.
Red books on species and habitats of European Concern: (http://www.mnhn.fr/ctn/redlist.htm)

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