UNITED NATIONS ENVIRONMENT PROGRAMME
MEDITERRANEAN ACTION PLAN

MED POL

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

LIGNES DIRECTRICES POUR L'ELABORATION D'INDICATEURS
D'ETAT ECOLOGIQUE ET DE REDUCTION DU STRESS

GEF

UNEP/MAP

Athens, 2004

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- Curbing Pollution
- Safeguarding Natural and Cultural Resources
- Managing Coastal Areas
- Integrating the Environment and Development

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FOREWORD

The riparian States of the Mediterranean Sea, aware of their responsibility to preserve and develop the region in a sustainable way, and recognizing the threat posed by pollution to the marine environment, agreed in 1975 to launch an Action Plan for the Protection and Development of the Mediterranean Basin (MAP) under the auspices of the United Nations Environment Programme (UNEP) and, in 1976, to sign a Convention for the Protection of the Mediterranean Sea against Pollution (the Barcelona Convention). The Convention entered into force in 1978 and was amended in 1995.

Recognizing that pollution from land-based activities and sources has the highest impact on the marine environment, the Contracting Parties to the Barcelona Convention signed in 1980 a Protocol for the Protection of the Mediterranean Sea against Pollution from Land-Based Sources (LBS Protocol). The Protocol entered into force in 1983 and was revised in 1996 to better cover industrial pollution sources and activities and to enlarge the coverage to include the hydrologic basin.

A Strategic Action Programme (SAP MED) to address pollution from land-based activities, which represents the regional adaptation of the principles of the UNEP Global Programme of Action (GPA) to address land-based pollution activities, was adopted by the Contracting Parties to the Barcelona Convention in 1997 as a follow up to the provisions of the revised LBS Protocol. The SAP MED identifies the major pollution problems of the region, indicates the possible control measures, shows the cost of such measures and establishes a work plan and timetable for their implementation.

In order to assist the Mediterranean countries in the long-term implementation of the SAP MED, particularly in the formulation, adoption and implementation of National Actions Plans (NAPs), a three-year GEF Project "Determination of priority actions for the further elaboration and implementation of the Strategic Action Programme for the Mediterranean Sea" was implemented by MAP, and in particular by the MED POL Programme, the MAP Regional Activity Centres and WHO/EURO. The project consists of numerous activities which include, among others, the preparation of regional guidelines and regional plans, whose main aim is to guide and assist countries to achieve the pollution reduction targets specified in SAP MED.

The present document is part of a series of publications of the MAP Technical Reports that include all the regional plans and guidelines prepared as part of the GEF Project for the implementation of the SAP MED.

TABLE OF CONTENTS

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 covers 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 misbelieves 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; Simboura and Nicolaidou, 2001)

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-Frangou, 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 l, 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.
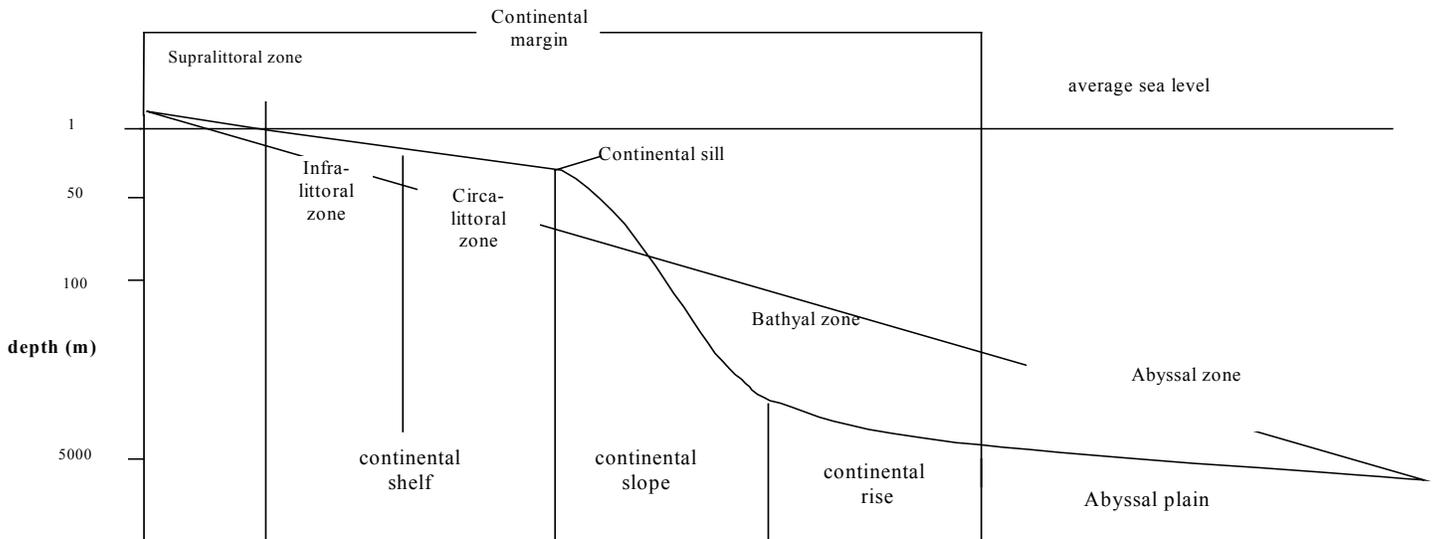
Figure 1. *Schematic presentation of the ecological benthic zones as proposed by Perés and Picard, 1964*

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 has 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
5. Habitat loss.

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 source 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). Indicators pertaining to the evaluation of contamination level via the study of coliphages in the waters, sediments, organisms are beyond the aims of this report.

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).

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. Measurements of Total Aromatic Hydrocarbons (PAH) in sediments and marine organisms are included in the workplan of the MEDPOL project which is implemented in most Mediterranean countries since the 80's.

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 which is however more intense in the North Mediterranean. Over-exploitation in the northern Mediterranean 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.

Habitat loss

Loss of coastal habitats is a major global concern. Besides coastal erosion related to a combination of human activities such as damning and coastal developments, wetlands and estuaries are threatened due to competitive use of the coastal zone but also subtidal areas, especially by trawling. The economic losses caused are substantial, but not generally appreciated. There are three main problems (Gray, 2003)
- Habitat degradation – from human waste (pollution);
- Habitat loss – leads to loss of species and the functions they perform;
- Habitat homogenisation – e.g. trawling converting many habitats to a single one.

Erosion data in the Mediterranean showed that the 1500km of artificial coasts can be found in the EU marine area (Balearic Islands, Gulf of Lyon, Sardinia, Adriatic, Ionian and the Aegean (with harbours and ports contributing the major part (250km). Based on the CORINE coastal erosion data about 25% of the Italian Adriatic coast and 7.4% of the Aegean Sea show evolutionary trends of erosion. (EEA, 1999a).

Corallium rubrum (photo: C. Bratits)

A well known case is that of the red coral *Corallium rubrum (see photo)* that is heavily exploited. However, its degradation is also attributed to reduction in water transparency due to pollution and turbidity.

Other known cases in the Mediterranean are that of loss of habitat of date mussel due to overexploitation, massive deaths of sponges (presumably attributed to diseases) and that of corals in the W. Mediterranean, Adriatic and N. Aegean.

If habitats are conserved then usually most species within the habitat are conserved and this is probably the simplest way to conserve biodiversity. Yet it is the mosaic of different coastal habitats that are important, collectively called the "landscape" or seascape".

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).

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

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[1]   FAO (the UN Food and Agriculture Organization).
[2]   OECD: Organization for Economic Co-operation and Development.

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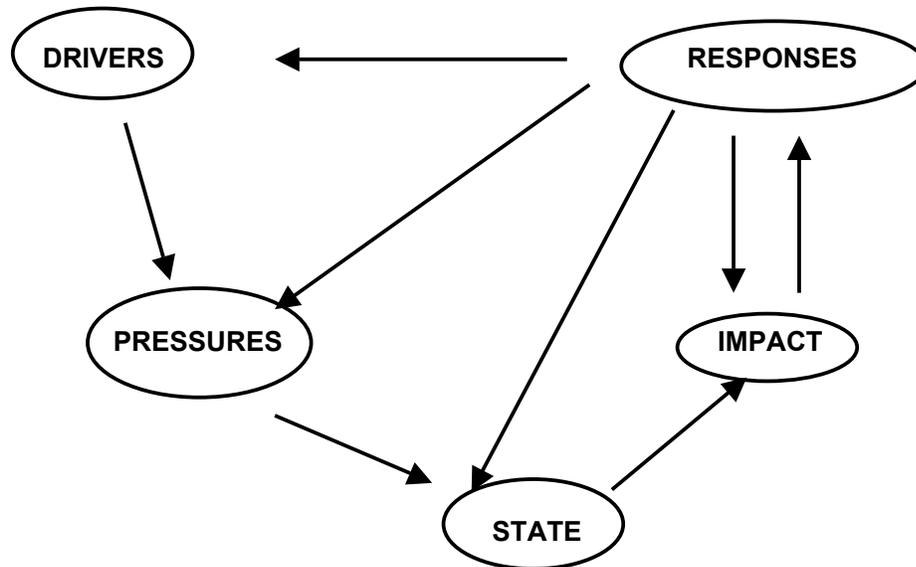
Figure 2. The DPSIR Framework for reporting on Environmental issues. Source: EEA, 1999b.

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

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[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.

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 is 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*

Recently 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.

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[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.   OVERVIEW OF 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 measurement 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.

Figure 3 presents graphically the various levels of biological organizations and clusters of indicators at these levels as well as the time frame within which these clusters are sensitive enough to provide info in the EcQoS.
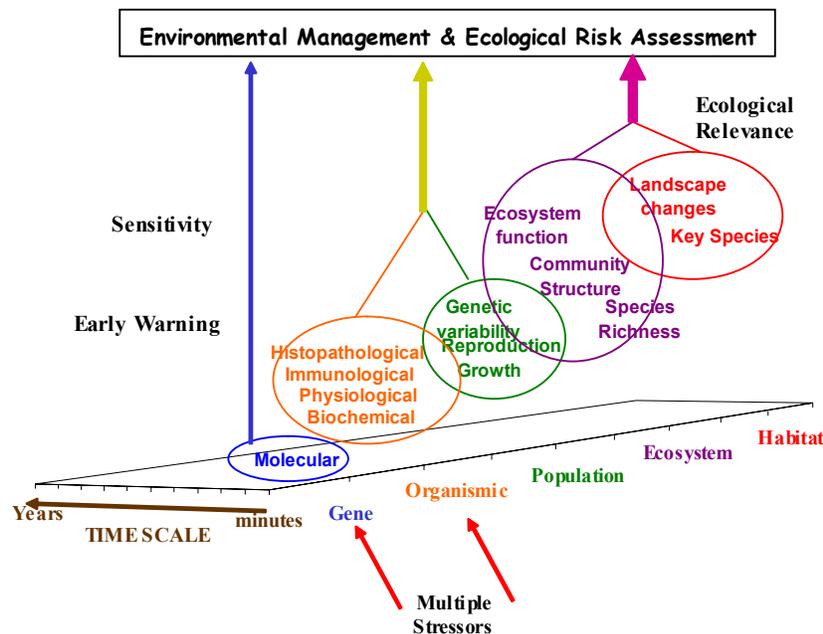
Figure 3.   Overall view of biological indicators (from gene to habitat) used to assess ECoQoS

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.

7.1 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 labelling 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.

7.1.1 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, labour intensive and require laboratories 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.

7.1.2 *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.

7.1.2.1 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.

7.1.3 *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.

7.1.4   *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.

7.1.5   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.

7.1.6   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.

7.1.7   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).

7.2 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
Potentially the mullet (*Mugil cephalus*) is a good indicator organism of contaminated sediments. It is a benthivore sedentary organism whose contact with the sediment is directly

favoured by the presence of contaminants in the environment. The use of some biochemical indices such as OFM (Oxygenases a function mixtes), SOD (superoxide dismutase), ADH (aldehyde dehydrogenase) and catalases constitute sensitive and specific monitoring tools for the assessment of environmental pollution.

7.3 Immunological Biomarkers

The response and functioning of the immune system is multifaceted. In many aquatic species the immunological system is particularly sensitive to immune-moderators, especially to xenobiotics. Their metabolism is susceptible to alterations which could be used as early warnings (indicators of stress and toxicity). 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, graft rejection, the percentage of circulating antibodies activated by lysozyme and the histology of the lymphoid tissue 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 but are sensitive to factors such as temperature, handling, transportation etc.

7.4 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 are cited in table 9.

Yet, much antibody-based recognition tests for specific proteins (e.g. cytochomes P450, stress proteins) can now be applied directly to histological 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).

Table 9

Advantages and limitations of employing histological markers to assess EcoQs.

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

Table 10

Proposed core set of biomarkers (UNEP/MAP Workshop, Athens, March 2003)

Biomarker	Observation	Enzymo/spectro	TYPE
Lysosomal stability	µ		
Lipofuschine	µ		
AchE			*
BPH			*
MTs			*
GST			*
CAT			*
MDA			*
Stress on stress			*
Macrophage activity	µ		*
Micronuclei	µ		
EROD (F)			*
Bile FACs (F)			*

NOTE: Among this list it's possible to select clusters to build.

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.

Table 11

Supplementary biomarkers proposed at UNEP/MAP workshop, Athens, March, 2003

Biomarker	Observation Antibody, Probe	Enzymo/	HPLC/Electro.	TYPE
Peroxisome	μ	*		
MDR	*		*	
CYPS	*		*	
Hormonal shift		*	*	
Embryo sex ratio	*			
Reproductive success	*			
Signal transduction	*		*	
Mitochondrial activity	*DNA alteration		*	*
Hsp				*
Gonadal alteration	μ			*
Apoptosis signals				*
Sperm parameters	μ	*		
Aromatase/Testosterone		*	*oxydative stress enzyme	*
VTG/VTG like		*		*

Table 12

Proposed biotests at UNEP/MAP workshop, Athens, March, 2003

Biotests	Water/ Column()	Intestice/ Water	Sediment	Chemical extracts	Type
Microtox	*	*		*	*
Algae	*	*			*
Mutatest/tox	*	*			*
Daphnia	*	*			*
Fish	*	*		*	*
Sea Urchin sperm		*		*	*
Mollusc larvae		*			*
DR-CALUX					*
ER-CALUX					*

(): Only working with highly polluted water

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 provides 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 respond 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 13.

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 13

**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 are 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).

The use of indicator organisms for the detection of anthropogenic change appears attractive to environmental managers, to the extent now that the term indicator generally implies a link to pollution studies (Wilson 1994). The WFD specifies the use of 'disturbance sensitive taxa' and 'taxa indicative of pollution' in its definition of ecological status of the benthic invertebrate fauna for both transitional and coastal waters.

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 (2002) 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 14) 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 14

Soft bottom community types proposed by Simboura and Zenetos (2002)

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.

NUMBER OF BENTHIC SPECIES (ZOOBENTHOS, PHYTOBENTHOS)
NUMBER AND ABUNDANCE OF EXOTIC SPECIES (ZOOBENTHOS, PHYTOBENTHOS, ZOOPLANKTON, PHYTOPLANKTON)
PRESENCE AND COVERAGE OF BENTHIC MACROPHYTES
PRESENCE/ABUNDANCE OF SENSITIVE AND/OR OPPORTUNISTIC BENTHIC SPECIES/TAXA (ZOOBENTHOS, PHYTOBENTHOS)
COMMUNITY DIVERSITY (H) (ZOOBENTHOS, PHYTOBENTHOS, ZOOPLANKTON)
BIOTIC INDEX: complex index incorporating ecological groups (ZOOBENTHOS, PHYTOBENTHOS)
TAXONOMIC DISTINCTNESS INDEX
POLLUTION INDEX (ZOOBENTHOS)
LEGISLATION CONCERNING RARE, ENDANGERED OR THREATENED MARINE SPECIES: ALL TAXA

COMPARISON OF DOMINANCE CURVES (ABC, W-statistics): ZOOBENTHOS
LOG-NORMAL DISTRIBUTION: ZOOBENTHOS
GEOMETRIC ABUNDANCE/SIZE CLASSES DISTRIBUTION: ZOOBENTHOS
THE RATIO BETWEEN r- AND K- SELECTED SPECIES: (ZOOBENTHOS, PHYTOBENTHOS)
INFAUNAL TROPHIC INDEX: based on the trophic status of the ZOOBENTHOS

PLANKTONIC COMMUNITIES
Phytoplankton
Phytoplankton parameters which are directly related to the availability of nutrients as well as the hydrographic conditions in a given ecosystem are widely used to define the eutrophication level but are not sensitive to define the low ecological quality due to other stressors.
Among the indicators mostly used are:
- Primary production: measured as gC/m^2-yr;
- Total biomass of phytoplankton which is measured as Chlorophyll concentrations;
- Dominance Index (details under *Description of Biological indicators*).

Zooplankton
Zooplankton, being the major consumer of the primary production, constitutes the food source of organisms at higher trophic levels. The parameters mostly studied are biomass, abundance and species composition. However, as these are influenced by the hydrographic conditions and are also season dependent, study of zooplankton in coastal areas in terms of the above parameters is not considered adequate to draw reliable conclusions on the ecological quality of a given area. On the contrary the identification of changes in species composition related to **long-term trends** in the ocean is a strategy used to monitor the influence of **global changes** on marine communities.

Besides the above, other zooplankton parameters occasionally used as status or impact indicators are:
- Average biomass of the jellyfish *Aurelia aurita* (g m^{-2});   impact
- Total number of species: Qualitative zooplankton studies describing, among others, the spatial distribution of the main copepod species in the western Mediterranean have shown **no big differences** in planktonic community structure (Fernadez de Puelles *et al.*, 2003);
- Number of neustonic copepods (family Pontellidae) (ind m^{-3})   state
- Number of Polychaeta larvae in total number of meroplankton (%) ?
- Specific production of dominant zooplankton species;
- Total Pelagic Biomass/Total benthic biomass ??
- Number and Biomass of *Noctiluca scilntillans* in the total zooplankton (%).

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) should 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 have 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 (Habitats 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 crustacea), followed by typical analysis of species composition and abundance.

INDICATOR
CHANGES IN THE DISTRIBUTION AREA OF HABITAT TYPES

List of Indicators

The tables that follow (Table 15, 16) include the indicators used for assessment of the EcoQ, which will be described in detail in the following chapter. Table 15 lists a core set of Indicators among those most widely used and successfully implemented at either local or national level uptodate. Complied list of Additional indicators (useful but second priority ones), some of them applicable at a verylocal level are presented in Table 16.

Table 15

Proposed core set of ecosystem indicators at UNEP/MAP workshop, Athens, March, 2003
(cc: cross-cutting among the biological elements: PP=phytoplankton, ZP= zooplankton,
PB=phytobenthos, ZB=zoobenthos)

Indicator	Taxonomy Group	Type
Changes in population of key species	CC	Impact
Occurrence of nuisance species (HABS)	Phytoplankton	Impact
Ecological evaluation index based on macrophytes (EEI)	Phytobentos	State
Number of benthic species	CC (PB, ZB)	State
Ecological quality index based on zoobenthos (Bentix)	Zoobenthos	State
Changes in the distribution area of habitats types	CC	Impact

Table 16

**Proposed set of supplementary ecosystem indicators
at UNEP/MAP workshop, Athens, March, 2003**

Indicator	Expertise	Taxonomic group	Type
Number and abundance of exotic species		CC (PP,ZP,PB, ZB)	Impact
Community Diversity		CC (PP,ZP,PB,ZB)	State
Dominance Index		Cc (phytoplankton /zooplankton)	State
Presence of sensitive/opportunistic benthic species		CC	State
Presence of sensitive/opportunistic zoobenthic taxa **Also described as Taxonomic Distinctness Index**		Zoobenthos	State
Comparison of dominance curves		Zoobenthos	state
Log-normal distribution		Zoobenthos	State
Geometric abundance/size classes abundance		Zoobenthos	State
The ration between r and K selected species		Zoobenthos	State
Infaunal trophic index		Zoobenthos	State
Taxonomic Distinctness Index		Zoobenthos	State
Pollution Index		Zoobenthos	
Plankton species composition - % composition of key groups (number and biomass) **Also described under Dominance Index**		CC (PP,ZP)	State
Total phytoplankton biomass (mg m^{-3})		Phytoplankton	state
Biomass of each phytoplankton species (mg m^{-3})		Phytoplankton	No
Density of each phytoplankton species (cells l^{-1})		Phytoplankton	No
Seasonal succession of key phytoplankton species (cells l^{-1})		Phytoplankton	No
Annual maximum density (cells l^{-1}) of each blooming phytoplankton species **Described above as Occurrence of nuisance species (HABS)**		Phytoplankton	impact
Total biomass of zooplankton (mg m^{-3})		Zooplankton	State/no
Total number and species composition)*		Zooplankton	No
Number of neustonic copepods (family Pontellidae) (ind m^{-3}) **Also under Specific production of dominant zooplankton species**		Zooplankton	state
Number of Polychaeta larvae in total number of meroplankton (%)**Also under Specific production of dominant zooplankton species**		Zooplankton	?
Specific production of dominant zooplankton species		Zooplankton	
Total biomass of phytoplankton/		Zooplankton	Eutrophication
Total Pelagic Biomass/Total benthic biomass		Zooplankton	?
Average biomass of the jellyfish *Aurelia aurita* (g m^{-2}) **Also under Specific production of dominant zooplankton species**		Zooplankton	Impact
Number and Biomass of *Noctiluca scilntillans* in the total zooplankton (%) **Also under Specific production of dominant zooplankton species**		Zooplankton	
Total primary production (mg Corg m-2 month-1)		Macrophytes	State
Total macrophyte biomass (mg m^{-2})		Macrophytes	
Biomass of dominant macrophyte species (g m^{-2})		Macrophytes	State
Key groups: Chlorophyta, Rhodophyta, Phaeophyta (%biomass)		Macrophytes	
Key genera (presence/absence) **Also under Presence of sensitive/opportunistic benthic species**		Macrophytes	State
Specific production of the dominant species of macrophytobenthos (d^{-1})		Macrophytes	
Legislation concerning rare, endangered or threatened marine species			

8. DESCRIPTION OF BIOLOGICAL INDICATORS TOWARDS ASSESSMENT OF ECOLOGICAL STATUS AND IMPACT

Changes in 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 (UNEP RAC/SPA, 1998c, UNEP RAC/SPA, 1999b, 1999c);
- Action plan for the protection of cetacean;
- Action plan for the conservation of marine vegetation in the Mediterranean Sea (UNEP RAC/SPA. 1999d.

In the stage of adoption are two more action plans: Action plan for the protection of cartilaginous fish and Action plan for the protection of invasive species (Barcelona 31.10.2002).

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 17). In addition the species whose exploitation is regulated (Table 18) 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 continuous values depending on the degree of stress, so as to assess directly the impact to stress or response to a managerial strategy.

Table 17

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. Official Journal L 322, 14/12/1999 pp. 0003 – 0017
(http://faolex.fao.org/docs/texts/eur18724.doc)

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

Table 18

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

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

Ecological Evaluation Index Based on Macrophytes (EEI)

Rationale: 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.

Definition: 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. A 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.* (2001) 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 (e.g. surface for the seagrasses, linear for the seaweeds).

Table 19

Functional characteristics and growth strategies of marine benthic macrophytes
**(after Orfanidis *et al.*, 2001)**

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

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).

**Number of Benthic Species (Zoobenthos, Phytobenthos)**

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 4.
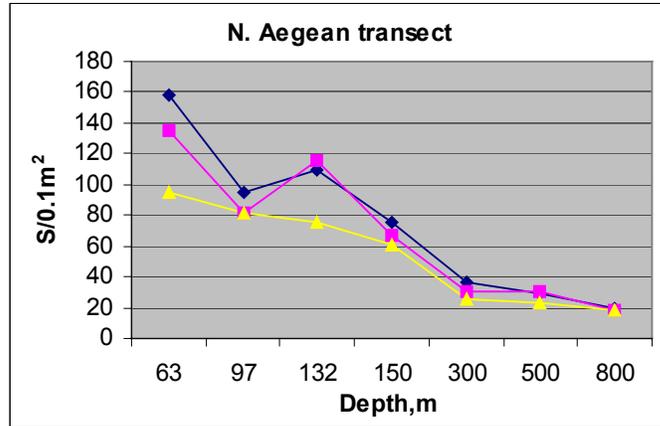
Figure 4.   Trend in zoobenthic species variety with depth. Lines correspond to replicates.
Source: NCMR, 2001

Sediment type is the second most significant factor influencing the species variety in a given biotope. In the two examples presented in Figures 5, 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.
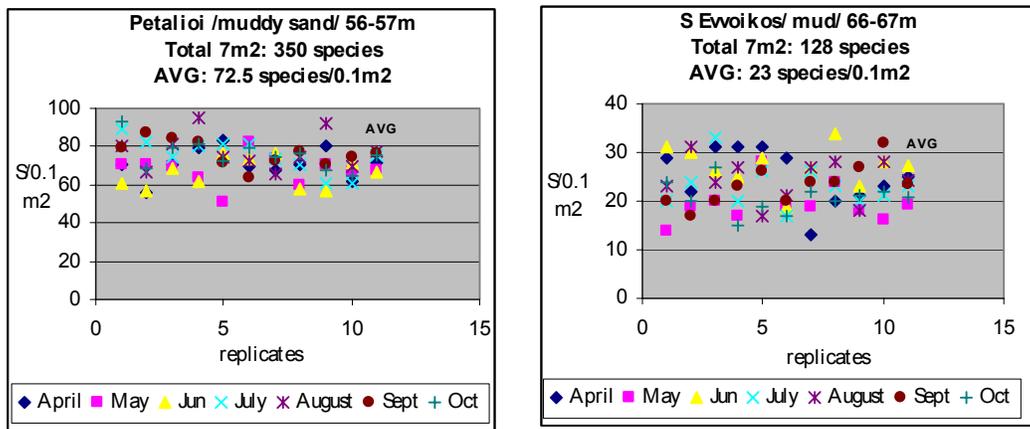
Figure 5.   Variation in species number per sampling unit $(0.1m^2)$ and monthly average $(0.1m^2)$
in different habitats (depth, sediment type). Data from TRIBE project (NCMR 1997)

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 6 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).
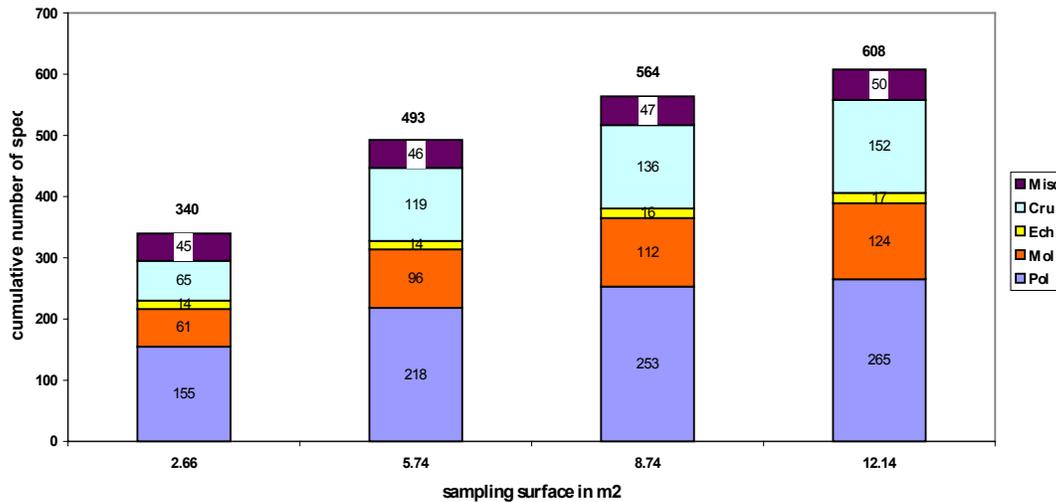
Figure 6.   Trend in species variety with sampling surface sampled/analysed.
Source: NCMR, 2001

An example of the effectiveness of S in ecological quality assessment is presented in Table 20. 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 20

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

Advantages: 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.

Limitations: 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).

Ecological Quality Index Based on Zoobenthos (Bentix)

This is a Biotic Coefficient designed (Simboura and Zenetos, 2002) to classify benthic communities to an Ecological Quality Status (EQS) according to the WFD requirements (EEC, 2000). It is based on the initial idea of Glemarec & Hily (1981) modified by Borja *et al.* (2000) and further modified to become more simple in its use and suit the Mediterranean benthic diversity.

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:

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

The bentix takes continuous values from 2 to 6, and equals 0 when the area/community is azoic. A classification system (Table 21) appears as a function of Bentix including five levels of ecological quality status (EQS).

Table 21

Classification of EcoQ according to range of the Biotic Index
(source: Simboura and Zenetos, 2002)

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

Advantages: The bentix 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 bentix 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 bentix 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 bentix 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 labelled 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.

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 22. To these we must add the endemic sponge species Petrobiona massiliana and Ircinia cheuvreuxi which consist important biotopes for many endobiotic, epibiotic, symbiotic organisms and can attain large densities (up to 409 ind./dm3: Rutzler, 1976). 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 22

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

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).

An index based on changes in coverage of macrophytes could be based on 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.

Supplementary indicators

Number and Abundance of Exotic Species (Zoobenthos, Phytobenthos, Zooplankton, Phytoplankton) including Harmful /Toxic macroorganisms

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 Mediterranean has a range of environmental conditions that allow the coexistence of species with much different ecological requirements, being very prone to be invaded: all the events of the past leave, thus, some memory. Coming to recent times, the arrival of some species, like gelatinous predators, shows that a single new species can change dramatically the functioning of a large ecosystem. The impact of new species cannot be predicted: Mnemiopsis is a harmless blob of jelly along the east coast of the United States, whereas it is a disaster in the Black Sea!

The invasion of the Mediterranean by exotic species has been mainly studied with regard to nektonic and macrofaunal/floral 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. Some of the species became a serious nuisance to ecosystem functioning, like *Rhopilema nomadica*, a large jellyfish that swarms regularly in the Eastern Mediterranean. Maybe it is not as harmful as *Mnemiopsis* in the Black Sea, but it has surely a great impact on coastal communities (and economies). (Galil & Zenetos, 2002).

Of the best known cases of introduced harmful exotic species in the Mediterranean is that of *Caulerpa taxifolia.* A small colony of *Caulerpa taxifolia* introduced into the Mediterranean in 1984 from a public aquarium (Oceanographic Museum of Monaco, where it was cultivated beginning in 1982) has spread to more than 6,000 hectares today, out competing native species and seriously reducing diversity in areas of the north-western Mediterranean. Yacht anchors and fishing gear have carried it from anchorage to anchorage and from harbour to harbour, sometimes over great distances (MedWaves, 1997, French N° 34, pp 11-13.)

Another algal species, The Erythrean *Caulerpa racemosa* has replaced, between 1992 and 1997, the native sea grass meadows of *Posidonia oceanica* in Moni Bay, Cyprus, and prompted significant change in the benthic macrofauna: the abundance of gastropods and

crustaceans decreased, whereas that of polychaetes, bivalves and echinoderms increased (Argyrou *et al.* 1999).

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.

With regard to planktonic organisms introduced via ballast tanks they are classified to one of the four general taxonomic groups: zooplankton, phytoplankton, bacteria, and virus-like-particles. A considerable variation exists in the concentrations of organisms arriving in unexchanged /untreated **ballast water** among vessels. Some of this variation is explained by (a) season and (b) voyage duration. Several studies also indicate that considerable variation exists among ballasting events, within the same port and season, which undoubtedly contribute to the observed variation.

The median concentrations of organisms estimated by a recent analysis of the ICES/IOC/IMO Study Group on Ballast Water and other Ship Vectors (SGBOSV) for unmanaged ballast water provide a useful frame of reference in consideration of ballast water standards.

Recognizing the inherent risk with any discharge, and the current concentrations delivered in untreated ballast water, SGBOSV recommends standards at least 3 orders of magnitude below the observed median concentrations for zooplankton and an equivalent or higher level of reduction for phytoplankton. (Gollasch and Raaymakers, 2003).

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.

The ICES/IOC/IMO Study Group on Ballast Water and other Ship Vectors (SGBOSV) is active in the field of exotics via shipping and has gathered expertise from Mediterranean countries (France, Italy, Greece).

Limitations: The CIESM atlases do not cover all groups.

Many areas such as N African coasts are literally unexplored.

Dominance Index (applied to phytoplankton, zooplankton)

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 planktonic 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

Advantages: 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.

The dominance index is one of the few measures which is successfully used in zooplanktonic studies as opposed to the overall zooplankton composition and density. Under extreme conditions zooplanktonic community can became almost monospecific (Siokou-Frangou *et al.*, 199: case of Elefsis bay).

Limitations: 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/Abundance of Sensitive/opportunistic Species

Definition: The presence of indicator species mentioned either as sensitive (fragile, and slowly reproducing) or opportunistic (small, short-lived, species). The presence can be expressed either 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: 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. 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 23) shows the zones of pollution with the respective opportunistic macrozoobenthic species in sandy-muddy communities. These are mostly polychaets.

Table 23

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.

Glemarec & Hily (1981) based on the sensitivity of zoobenthic species to an increasing stress gradient have grouped them into five clusters. The first cluster (Group 1) includes species very sensitive which are present only under unpolluted conditions whereas Group 5 includes opportunistic species which dominate in heavily impacted ecosystems.

Simboura and Zenetos (2002) have compiled a preliminary list of sensitive taxa according to community type. Based on the presence and relative dominance of amphipod populations which are classified among the sensitive species and the degree of pollution in hard substrata three groups of species have been defined (Table 24) corresponding to three classes of ecological status from very pure to polluted (Bellan-Santini, 1980). 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 25.

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 24

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 25

**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.* (2001) 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: 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).

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

Presence of Sensitive/opportunistic Zoobenthic Taxa (Also measure of Distinctness)

Taxonomic Distinctness Index

Rationale: Benthic taxa indicative of environmental disturbance have been reviewed by Rygg (1995), Borja *et al.* (2000) and in the Mediterranean by Orfanidis *et al.* (2001) [phytobenthos] and Simboura and Zenetos (2002) [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.

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). **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). 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).

Taxonomic distinctness (Δ^+) is a univariate (bio) diversity index. It utilises simple species lists (presence/absence data) to derive Δ^+ which encompasses not only distribution of abundance's amongst species but also the taxonomic relatedness of the species. This index is defined as the average taxonomic path length between any two randomly chosen species, traced through a Linnean or phylogenetic classification of the full set of species involved.

The weighting of steps in the taxonomic hierarchy involves taking the species list for each locality and/or habitat and calculating an average taxonomic distinctness index namely,

$$\Delta^+ = [\Sigma\Sigma_{i <j} \omega_{ij}] / [m(m-1)/2]$$

Where: m= number of species in the particular study, ωij= the weight (path length) given to the taxonomic relationship between species *I*, *j*. Δ^+ = average path length between any two randomly chosen species from the study.

Weights (v) are assigned to each section of the path, the step linking one taxonomic level to the next coarsest division. Clarke and Warwick (1999) have experimented with different weighting whereby various taxonomic levels (species, genera, families, suborders, orders, subclasses and class) are given different path weights.

An appropriate variance formula is derived from other analysis, which leads to a 'confidence funnel'. This enables the user to check Δ^+ of their samples and to assess whether a locality has a 'lower than expected' average taxonomic spread. In degraded habitats the Δ^+ values will fall below the lower boundary of the 'funnel'.

Advantages
- It is robust to variation in sampling effort;
- There is a statistical framework for assessing samples departure from 'expectation';
- Appears to decline monotonically in response to environmental degradation whilst being relatively insensitive to major habitat differences;
- It utilises any simple species lists (presence/absence data) which reduces sorting time;
- Related to trophic diversity, but unlike species richness measures it is independent of habitat type. Consequently, it's a powerful index that could allow the comparison of dissimilar sites e.g. sand and muds;
- Applied to a range of conditions e.g. pristine, polluted, reduced salinity conditions;
- Proved informative in other studies including benthic meiofaunal, macrofaunal and groundfish studies.

Disadvantages:

- unable to distinguish the 5 classes.

Community Diversity (H) (Zoobenthos, Phytobenthos, zooplankton, phytoplankton)

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 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 successional 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 both 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 (2002) 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 26 presents the range of diversity values estimated per community type.

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 27. An effort to classify Greek Waters with a similar approach was attempted by Zenetos and Simboura (2001).

Table 26

**Range of Community diversity (H) according to sampler (0.05. 0.1, 0.2, 0.5m^2)
and community type (after Simboura and Zenetos, 2002)**

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)

Table 27

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

	Classes				
	I	II	III	IV	V
Parameters	Very Good	Good	Fair	poor	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.

Zooplankton
The evolution of community diversity index in polluted and undisturbed zooplanktonic communities suggests a different community structure among the differently impacted areas. As such the H has been used in the Mediterranean by Siokou-Frangou & Papathanassiou (1991): Greek coasts; Scotto di Carlo & Ianora (1983): Italian coasts; Patriti, (1984): French coasts. However, up-to-date there ha not been any classification scheme to assign the pelagic ecosystem to one of the 5 WFD classes (Siokou-Frangou, pers. Commun).

Phytoplankton
Community diversity of phytoplankton assemblages has been widely used to assess ecological quality (i.e. in Tunisia in the marine farms for molluscan culture as well as in the Gulf of Gabes). But as in zooplankton not assigning environmental quality into one of the five levels system.

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.* 1997a and b).

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)

The ITI was developed as an aid to identify changed and degraded environmental conditions as a result of organic pollution e.g. in sewage sludge disposal grounds, sewage outfalls and oil drilling by-products.

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 meanings 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.

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

<u>Limitations</u> (from Miles, A. & Price, N., 2002)
ITI is not applicable in transitional Waters.
ITI was designed for use in coastal waters with organic contamination, only.
ITI values are assigned to classify areas of seabed into 3 classes: Normal, Changed, Degraded.
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.

Benthic Pollution index (BPI)

Rationale. The Benthic Pollution Index uses an approach developed by Leppakowski (1975) for the Baltic. For this index, areas of the estuary are assigned a score and then placed in to one of three groups. According to their observed response to organic pollution,
BPI can be calculated using the formula. **BPI = 10- (5x +4y +2 (z – a /2))/ a.**

Where: a = whole area, x = area of secondary minimum, y = area of secondary minimum, z = area of the primary maximum. An undisturbed area will display a BPI of 10.

Advantages:
Valid in the Baltic Sea, which has few species.

The index has also only been shown to be successful in coastal waters.

Limitations:
Groups are typed according to their response only to organic pollution.
High level of taxonomic expertise required.
Validaton in Sea areas with rich species diversity is questioned.

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 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 — 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.

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[1] Unternational Union for the Conservation of Nature.

<u>Limitations:</u> 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.

Specific production of dominant zooplankton species

During the 1960-1969 period, the average biomass of the main zooplanktonic organisms in the upper 100 m was 308 mg/m^3, this increased to 471 between 1970 and 1975 and again rose to 527 mg/m3 between 1976 and 1981. However, the increase was entirely due to two gelatinous species; the dinoflagellate Noctiluca scintillans and the ctenophore Pleurobrachia rhodopis (58, 67). A similar increase in the zooplankton was observed in the western Black Sea between 1978 and 1980. In the upper 100 m the zooplankton biomass increased in offshore areas (water depth >200m) by 1.7-2 fold, at the northwestern shelf the increase was 3-4 fold, in shallow coastal areas (0-25m) this rose to 10 fold. These changes were caused exclusively by the increase in Noctiluca scintillans (59). This corresponds with a significant increase in the phytoplankton biomass in the northwestern Black Sea (142).

In the area off the Danube estuary (in the upper 100 m) the average zooplankton abundance and biomass increased by factors of 3 and 5 respectively from the 1970s to the 1990s (63). As for other areas the proportions of Noctiluca and Acartia clausi also increased significantly. (Kovalev *et al.*, 1999).

Large scale changes to trophic structure, particularly if fishing of larger predatory species is not controlled, may result in that small pelagics become the major components of fish biomass. Opportunistic gelatinous species tend to be more aggressive feeders, at the high end of the food chain, than larval fish competing for the same food resources.

In the late l960s and 1970s, conditions in the Black Sea enabled its numbers to swell to such an extent that it proved a great nuisance on area beaches.

Outburst of the Aurelia aurita. Total biomass in the Black Sea reached up 300-500 million tons.

Outburst of the Mnemiopsis. Its total biomass in the Black Sea reached 700 million tons.

The EROS (European River/Ocean System) team has set up a data base from existing physical and biogeochemical data and attempted to develop a mathematical model capable of predicting the response of the coastal ecosystem to natural changes in the environment and human impacts.

For the first time, studies of the relationship between plankton communities and nutrient availability were combined with studies of the feeding behaviour of important planktonic animals. EROS analysis has revealed that the explosive development of jellyfish is the consequence of pollution and overfishing (EROS, 2000). Increased nutrient inputs into the Black Sea, due to changes in farming practices, combined with increases in industrial activities and population density, have stimulated plankton growth and related fish productivity. This, in turn, has stimulated the growth of opportunistic gelatinous species.

Two members of the scyphozoan phylum that have made dramatic appearances in the Black Sea are *Rhizostoma pulmo* and *Aurelia aurita. R. pulmo,* mentioned earlier, is native to the Mediterranean, North Sea, Black Sea, and Sea of Azov. Feeding on zooplankton plus the eggs and larvae of fish and shellfish, this jellyfish was not formerly considered a big player in the Black Sea's animal community. In fact, in some parts of its range, this jellyfish was considered beneficial as it offered protection to certain fish larvae and served as food for sea turtles.

9.   INDICATORS ACCORDING TO HUMAN ACTIVITIES 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 28 while the effects of sewage sludge disposal are seen on Table 29.

Table 28

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 richness, 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)

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 (i.e. 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 noteworthy 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 29

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 allow 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.

Table 30

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

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 30. 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 31). 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 31
Effects 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 (Distance from discharge point)	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

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 32). 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, recession 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 32
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 -Impairment of growth and reproduction-death of biota -Physiological and behavioural abnormalities -Greatest impact on intertidal and subtidal zones -Acute or chronic toxic effects on planktonic 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 opportunists (NCMR, 2001). -in seaweeds short-term growth reduction in intertidal species (Lobban and Harrison, 1994) -in seagrasses no direct effect -shift in planktonic 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 33. 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 (Côte 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 33

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
Carbomates	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 34).

Table 34

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

Impact on:		Suggested indicator
Bird population changes (food changes)		
Bycatch (unwanted) of mammals	Monk seals, turtles 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 35).

Table 35

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).

An inventory of the fauna and flora in ports and lagoons and subsequent monitoring of the biota (mostly planktonic organisms incdin bacteria) been proposed as the only way to realise slow coming changes that may have catastrophic effects

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 sought 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 algae 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: According to the Tunisian National Environmental Agency, long term studies along the Tunisian coasts include monitoring of phytoplankton (REPHY); microbiological studies (REMI); chemical analyses (RENOC); chemical, microbiological and toxicological monitoring in the MEDPOL framework; monitoring of bathing waters. Considerable work at national level is conducted with regard to chemical and physical parameters while there is accumulated experience (MEDPOL, COPEMED) in the study of bivalves as bioindicators. Moreover exotic species of Atlantic or Erythrean origin have been recently investigated (fax by K.Attia, 13.5.03).

Studies concerning indicators among benthos are very limited in Tunisia, although the expertise is there (fish, sponges, molluscan, crustacean, reptiles and macroalgae). No national monitoring system for EcoQs with the exception of some research on *Posidonia* in some areas receiving industrial effluent 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 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 36). Within the framework of MEDPOL 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 36
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 turbinate*	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 decussates*	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 7). 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.
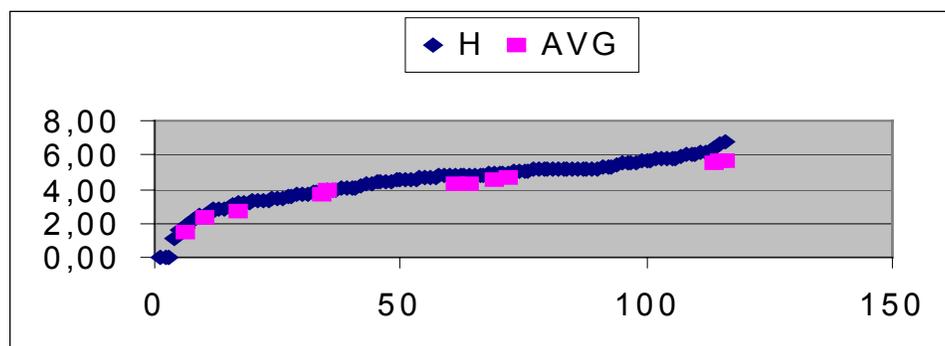
Figure 7. Distribution of community diversity (H) over 116 Greek sites. AVG: H/0.1m2 (after Zenetos and Simboura, 2001)

An even more precise division, yet somewhat arbitrary, has been suggested by Simboura and Zenetos (2002), 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:	$H \leq 1,5$: Azoic to very highly polluted –examples from Elefsis Bay, Thessaloniki
poor:	$1.5 < H \leq 3$: highly polluted – examples from Saronikos, Thermaikos
moderate:	$3 < H \leq 4$: moderately polluted
good:	$4 < H \leq 5$: for transitional zones
high:	$H > 5$: reference sites

Case study: oil spill accident

Table 37

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)	$23/0.1m^2$	$8/0.05 \ m^2$	$16/0.05 \ m^2$	$17/0.05 \ m^2$

Case study: dumping

An example of the effects of dumping on benthic communities within the Mediterranean follows below (Table 38).

Table 38

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	$30 \ (0.2 \ m^2)$	1797	3.76	0.78
Reference values		$70 \ (0.2 \ m^2)$	1585	5	0.87

Case study: number of molluscan exotics

Oyster farming in Mediterranean coastal waters (Adriatic, Etang de Thau) 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 8)
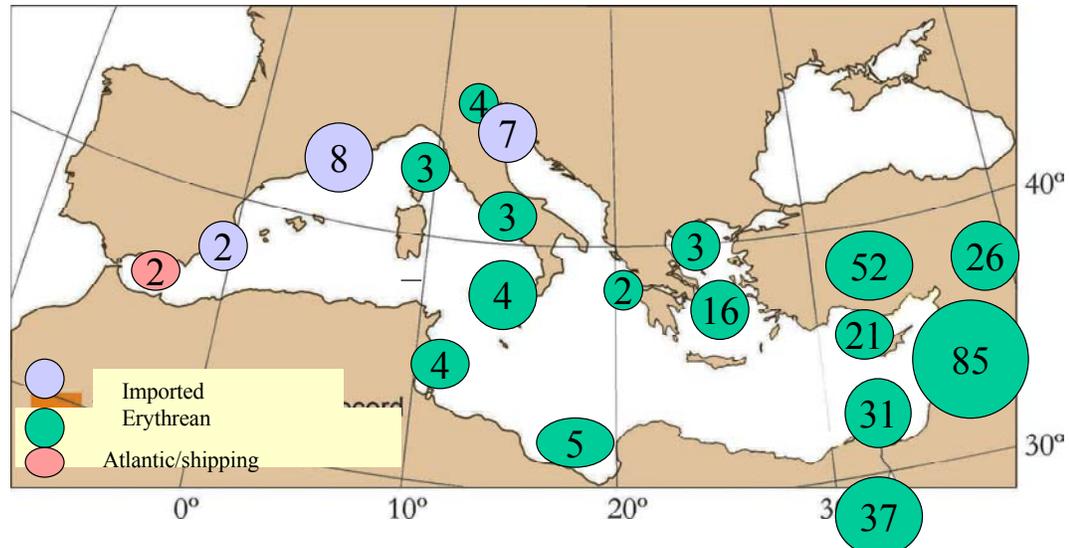
Figure 8.   Distribution of exotic mollusca in the Mediterranean up to December 2000: Indo-Pacific origin (green), Atlantic (pink) and introduced via aquaculture (grey).
Source: CIESM (www.ciesm.org/atlas/)

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:

Indicator: abundance of sensitive taxa.

Objective of developing: it is to keep high abundance and species variety of sensitive taxa.

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

How do we measure it? 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).

Under the EU Marine Strategy the EMMA (European Marine Monitoring and Assessment) group has been set up. The group will look at the similarities between work being carried out for WFD purposes and work taking place within the marine conventions. The first meeting of this group takes place in October 2003.

12. THE WAY FORWARD

There is a need of basic information. The existing data concerning biodiversity must be located. Coordination 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 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 forwards 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 constitutes 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 vertical 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 "Marine Protected 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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