



# **Mediterranean Marine Science**

Vol 3, No 2 (2002)



 **Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index**

 *N. SIMBOURA, A. ZENETOS* 

doi: 10.12681/mms.249

# **To cite this article:**

SIMBOURA, N., & ZENETOS, A. (2002). Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index. *Mediterranean Marine Science*, *3*(2), 77–111. https://doi.org/10.12681/mms.249

#### *Mediterranean Marine Science*

Vol. 3/2, 2002, 77-111

# **Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index**

#### **N. SIMBOURA and A. ZENETOS**

National Centre for Marine Research, Institute of Oceanography, P.O.712, 190 13 Anavissos, Attiki, Greece

e-mail: msim@ncmr.gr

#### **Abstract**

*A general scheme for approaching the objective of Ecological Quality Status (EcoQ) classification of zoobenthic marine ecosystems is presented. A system based on soft bottom benthic indicator species and related habitat types is suggested to be used for testing the typological definition of a given water body in the Mediterranean. Benthic indices including the Shannon-Wiener diversity index and the species richness are re-evaluated for use in classification. Ranges of values and of ecological quality categories are given for the diversity and species richness in different habitat types. A new biotic index (BENTIX) is proposed based on the relative percentages of three ecological groups of species grouped according to their sensitivity or tolerance to disturbance factors and weighted proportionately to obtain a formula rendering a five step numerical scale of ecological quality classification. Its advantage against former biotic indices lies in the fact that it reduces the number of the ecological groups involved which makes it simpler and easier in its use. The Bentix index proposed is tested and validated with data from Greek and western Mediterranean ecosystems and examples are presented. Indicator species associated with specific habitat types and pollution indicator species, scored according to their degree of tolerance to pollution, are listed in a table. The Bentix index is compared and evaluated against the indices of diversity and species richness for use in classification. The advantages of the BENTIX index as a classification tool for ECoQ include independence from habitat type, sample size and taxonomic effort, high discriminative power and simplicity in its use which make it a robust, simple and effective tool for application in the Mediterranean Sea.*

**Keywords:** Benthic indicators, Ecological quality, Biotic index, Mediterranean, Greece.

# **Introduction**

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. Yet, biological diversity, extending from gene to

direct chemical analyses of water and sediment, which are usually very sensitive and accurate, do not necessarily reflect the actual ecological state, for several reasons (for a detailed discussion see PHILLIPS & RAINBOW, 1994).

The impacts of human activities on the

ecosystem, are most evident in coastal areas. Besides eutrophication, 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.

At the European level, the development of biological indicators, as a tool for the knowledge of the environment and hence the protection of biological diversity of coastal and marine ecosystems has been advanced through the implementation of the HABITATS directive, the biological quality elements of the Water Framework Directive (WFD), the Integrated Coastal Zone Management (ICZM) proposal, the Bathing Waters Directive and others. Moreover, the European Commission is funding several initiatives such as the European Platform for Biodiversity Research Strategy (EPBRS), the BIOMARE concerted Action, and Research projects in the framework of which the development of marine biodiversity indicators are key issues.

Among the biological quality elements for the definition of ecological status in coastal waters in WFD are the *Composition and abundance of benthic invertebrate fauna.* A general evolutionary pattern of the macrobenthic 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 & ROSENBERG (1978), and in the Mediterranean by PERES & BELLAN (1973).

Zoobenthos, through the long history of Mediterranean research, has been tested to be a biological element which can be reliably used for the classification of coastal and transitional water bodies. This is due to the stability and consistency of community structure and composition under given natural conditions, the uniformity of the various types of habitats encountered throughout the Mediterranean ecoregion, and the responsiveness to major environmental or anthropogenic changes.

An early discussion on the derivation and value of Ecological Quality Standards (EcoQS) and Objectives (EcoQO) is given by ELLIOTT, (1996). In the North Sea, measurement of EcoQ has focused on the state of benthic communities based on the two community attributes: species diversity, and community structure and functioning (De BOER *et al.,* 2001).

The Mediterranean coastal zone has seen accelerated urban development and industrialization in recent years. Both domestic and industrial waste are becoming an increasing threat to coastal marine habitats. Recent literature pertaining to the response of benthic communities to the increased stress in the Mediterranean region (ROS & CARDELL, 1991; CARDELL *et al.,* 1999; LAMY & GUELORGET, 1995) have highlighted the usage of benthic community parameters in defining short or long-term changes (SALEN-PICARD *et al.,* 1997). In order to conserve these environments, sensitive and reliable models must be developed and calibrated for monitoring marine pollution.

The development and choice of the most appropriate tools for treating and evaluating benthic data for the scope of typology and classification processes is essential. Specifically, the need to proceed towards conforming with the requirements of the EU Framework Directive for the Water Policy (EEC, 2000), was the initial drive to review and reevaluate the use of benthic indicators and to investigate possible use of novel indices. This work presents the development of a new biotic index based on the initial idea of BORJA *et al.*(2000) to combine the percentage abundances of five ecological groups in a single formula resulting to a series of numeric values. The novelty of this new index called BENTIX lies in the idea of treating benthic species as belonging to two wider ecological groups, the sensitive and the tolerant ones, thus reducing the number of the ecological groups used in the formula from five to actually two. This reduction (without jeopardising reliability) aims to simplify the

whole procedure avoiding at the same time the uncertainty of assigning species to one of five ecological groups. The paper also presents a reevaluation of the use of other benthic indicators as the diversity and species richness indices and compares them with the Bentix index. Finally, the paper provides a guidance towards the implementation of the requirements of WFD following all steps from the typology testing to classification using the descriptive tools of the macrozoobenthos quality element, all from a global viewpoint figuring the whole Mediterranean Sea.

#### **Materials and Methods**

Macrozoobenthic data compiled for this work concern the whole Mediterranean Sea and especially the Greek seas. Figure 1 shows the relationship among the various steps taken towards achieving the definition of the ECoQ in the Mediterranean ecoregion by using the macrozoobenthos element. Macrozoobenthos as a biological element comprises of sensitive and tolerant species according to their degree of tolerance to stress factors. Sensitive species are used for the characterisation of the habitat



*Fig 1:* Flow diagramme of typology and classification processes using benthic invertebrate fauna.

type and hence the testing of the typological definition of a given water body. Both sensitive and tolerant species are taken into account in the calculation of the Bentix index and the biological indices of Shannon diversity H' (SHANNON-WEAVER, 1963) and the species richness (S). The BENTIX index leads directly to the assessment of ECoQ if used alone, while the biological indices reach this goal only if taking into account the description of the habitat type related to typology. Finally both tools help to the assessment of the Ecological Quality Objectives (EcoQO) through the work of classification. Each step of this scheme is explained separately below:

#### *a) Definition of benthic indicator species*

Indicator organisms are species picked for their sensitivity or tolerance to various parameters, historically pollution. Benthic indicator species are used to a) define the habitat type (testing of typology) and b) classify the ecological quality. Based on the literature, zoobenthic species can be grouped into two major groups: the sensitive ones characterising a habitat type by their dominance (% abundance) or their exclusive presence in the specific habitats; and the tolerant: these are the so called resistant species and the first grade or second grade opportunistic ones. The indifferent species not affected by any kind of disturbance may be included in the first group. So in the general scheme presented, the sensitive species are used to define the habitat type and hence justify or test the typology of a given water body and both groups (the total fauna) are used in the plotting of the Bentix Index as well as the biological indices. The sensitive species are well adapted to their specific environment (k-strategy species) and are usually linked to one or a few similar habitat types presented in Annex I. Some are typical of a given type by their exclusive appearance in this type and others show a preference in a given type expressed by their numeric dominance. To give an indication of the

importance of these species within each habitat type, the highest percentages met in Greek or other Mediterranean ecosystems are given also in the tables of Annex I. The tolerant species are generally opportunistic with low ecological requirements but sometimes they present affinities with specific conditions. A compiled list of tolerant species or instability indicators is presented in Annex II.

# *b) Definition of the major benthic coastal and transitional habitat types encountered over the Mediterranean area.*

The next step was the definition of the major soft bottom habitat types in the Mediterranean through experience and thorough review of the literature. Defining the habitat type is one of the basic links serving as a biological testing of the typological definition of a given water body. This is essential for the knowledge of the environment and for evaluating the benthic indicators (especially the indices of diversity) during the process of classification. Table 1 presents the major benthic habitats encountered in the Mediterranean considering the main environmental and biotic factors (depth, type of substratum, biota). The sensitive indicator species presented in Annex I are used to associate a benthic community to one of the habitat types. It is noteworthy that some habitat types as the muddy bottoms or the lagoons normally host several tolerant species.

Habitat types are derived initially from an adjustment of the classical bionomical system of biocoenoses described by PERES & PICARD (1964) which applies for the whole Mediterranean and Europe. In this work these benthic habitats will be referred to as in table 1, giving also an alternative description and the original definition. These modifications have been made to adjust the original definitions to the local conditions; also to simplify and generalise the scheme for the purpose of this work which would be applicable at European level. For example the Coastal

#### **Table 1**

**Abbreviations used: VTC=Coastal Terrigenous muds. LEE=Eurythermal, euryhaline biocoenosis (met in lagoons and estuaries). SFBC= Fine well-sorted sands. SFHN= fine surface sands. SGCF=coarse sands and fine gravels under the influence of bottom currents. SVMC=calm water muddy sands. AP=photophilous algae. DC=Coastal detritus bottoms. C=Coralligenous.**



Terrigenous Muds (VTC) biocoenoses originally is described from the Circalittoral zone1. However, in Greek ecosystems it is usually met also in closed gulfs of shallower depths encountered in 20-30m. So, for the purpose of this work it shall be called as the habitat of muds. Generally, the scheme adopted for the classification of habitats into types is based on the factors of depth, type of substrate, and phytal cover.

#### *c) Development of a Biotic index (BENTIX)*

Soft bottom macrobenthic communities respond to environmental disturbance or stress by means of different adaptive strategies. GRAY (1981a) considers that pollution effects can be separated into two categories: disturbance and stress, but in this work this distinction is not taken into account and both

disturbance and stress, are considered as forms of pressure or pollution. Many authors have summarised the adaptive strategies towards pollution into three (GRAY, 1979) four (SALEN-PICARD, 1983) and five ecological groups according to their sensitivity to an increasing stress gradient (HILY, 1984; GLÉMAREC, 1986; MAJEED, 1987; GRALL & GLÉMAREC, 1977; BORJA *et al.,* 2000). Among these authors BORJA *et al.,* (2000) proposed a biotic index receiving continuous values as a function of the relative abundance of the ecological groups present. The formula of BORJA *et al.,*(2000) was based on the percentages of five ecological groups weighting their contribution with use of different factors. The concept of the new Bentix index lies in the reduction of the ecological groups involved in the formula, in

*<sup>1</sup> Circalittoral is defined as the bathymetric zone with upper limit the lowest extent of the marine angiosperms (around 35m) and with lowest (deepest) limit the lowest extent of sciaphilic algae (70-120m).*

order to avoid errors in the grouping of the species, and reduce effort in calculating the index, without at the same time loosing its discriminative power or sensitivity.

Towards this scope, the five different degrees of sensitivity or tolerance to disturbance factors described so far, were integrated based on their affinity, in three ecological groups described below. Also an attempt was made to compile a list of indicator species assigning a score ranging from 1-3 corresponding to each one of the three ecological groups (Annexes I, II). The information to classify the species into the ecological groups was derived from works providing ecological characterisation of species (BORJA *et al.,* 2000; CORBERA & CARDELL, 1995; SIMBOURA & NICOLAIDOU, 2001) and from reviewing the literature cited in Table 2.

# *Ecological groups*

*Group 1 (GI)*. This group includes species which are **sensitive** to disturbance in general. These species corresponds to the k-strategy species, with relatively long life, slow growth and high biomass (GRAY, 1979). Also species indifferent to disturbance, always present in low densities with non-significant variations with time are included in this group, as they cannot be considered as tolerant by any degree. Species belonging to this group were assigned with the score 1.

*Group 2 (GII).* Species **tolerant** to disturbance or stress whose populations may respond to enrichment or other source of pollution by an increase of densities (slight unbalanced situations). Also this group includes **second-order opportunistic** species, or late successional colonisers with r-strategy: species with short life span, fast growth, early sexual maturation and larvae throughout the year. Species belonging to this group were assigned with the score 2.

*Group 3 (GIII).* This group includes the **first order opportunistic** species (pronounced

unbalanced situations), pioneers, colonisers, or species tolerant to hypoxia. Species belonging to this group were assigned with the score 3.

Based on the above description it appears that species may belong to **one** "sensitive" group or **three** different "tolerant" subgroups: the tolerant species, the second order and the first order opportunistic species. In the procedure of integrating all degrees of sensitivity in the formula, the different subgroups should be weighted equally in relation to the others so as not to under- or overestimate a given subgroup's significance in the calculation of the index.

The maximum reduction of the ecological groups involved in the formula would result to two groups: one "sensitive" and one "tolerant" group which according to the above analysis, should relate with a quantitative ratio of 1:3.

The limits of the scale were set from 2 to 6 (being 0 when the sediment is azoic) reversing the scale of BORJA *et al.* (2000) in an ascending way so as the value of 6 will correspond and reflect high EcoQ or reference conditions concerning the macrozoobenthos biological element. Therefore the factor assigned to the sensitive group GI should be 6, so that if 100% of the fauna are sensitive species the value of the index should correspond to the higher limit of the scale. Also the ratio of 1:3 should be reversed to 3:1 in favour of the sensitive group corresponding to the upper limit of the scale. Consequently the "tolerant group" should be assigned with the factor 2 following the ratio of 3:1. In this way groups GII and GIII, although they may appear in the formula to give a better reflection of the fauna's composition, they are treated actually as one "tolerant" group. The resulting formula tested and validated with data from Greek communities as well as Mediterranean, is:

#### BENTIX = {  $6 X \%$  GI +  $2 X \%$  GII +  $+$  % GIII)}/100

Use of the Bentix can produce a series of continuous values from 2 to 6, being 0 when

**Table 2 Data sets used for designing, testing and validating the methods proposed.**

<b>REGION</b>	<b>REFERENCE</b>
Algeria	Grimes & Gueraini, 2001; Bakalem, 1981; 2001
<b>Adriatic Sea</b>	Ivesa et al., 2001; Zavodnic et al., 1981; Marano et al., 1989
Amvrakikos Gulf (Ionian Sea)	Bogdanos et al., 1989
Atalanti Bay (N. Evvoikos Gulf)	Zenetos et al., 1991
Catalan coasts	Desbruyeres et al., 1972-73
Cephalonia isl. (Ionian Sea)	Pancucci-Papadopoulou, 1996
Criti continental shelf (Aegean Sea)	Karakassis & Eleftheriou, 1997
Diavlos Oreon Straits; Chalkis,	<b>NCMR, 1992</b>
Maliakos Gulf	
Elba isl. (Western Mediterranean)	Bianchi et al., 1993
French coasts (Western Mediterranean)	Bellan-Santini, 1980; Masse, 1998; Gremare et al., 1998;
	Picard, 1965
Evros Delta (N. Aegean)	Gouvis et al, 1997
Geras gulf (Lesvos isl.)	NCMR, 1990; Pancucci-Papadopoulou, 1996
Gialova lagoon (Ionian Sea)	Koutsoubas et al., 2000
Iberian Atlantic coasts	López-Jamar et al., 1987
<b>Izmir</b> Bay	Cinar et al., 2001
Kalamitsi (Ionian Sea)	Zenetos et al., $\overline{1997}$
Kavala gulf (N. Aegean)	Papazacharias et al., 1998
Korinthiakos (Itea, Antikyra) (Aegean)	<b>NCMR, 1994</b>
Kyklades plateau (Milos isl.) (Aegean)	<b>NCMR, 1989</b>
Ligurian Sea (Western Mediterranean)	Albertelli & Fraschetti, 1995
Logarou, Tsoukalio, Rodia lagoons	Reizopoulou et al, 1996
Prado Bay (Gulf of Lion)	Massé, 1971
N. Evvoikos Gulf (Aegean)	NCMR, 1998, 2001b
Orbetello lagoon (Tyrrhenian Sea)	Morgana & Naviglio, 1995
Pagassitikos Gulf (Aegean)	<b>NCMR</b> , 2000a
Papas lagoon (Ionian Sea)	<b>NCMR, 2000b</b>
Patraikos Gulf	Pancucci-Papadopoulou, 1996
Rhodes isl. (SE Aegean)	<b>NCMR, 1987</b>
S. Evvoikos Gulf, Petalioi Gulf, Oropos	Simboura et al., 1998; NCMR, 2001c
Saronikos Gulf (Aegean)	Simboura et al., 1995b; NCMR, 1997; 1999; 2001c.
Sporades isl. (Aegean Sea)	Simboura et al., 1995a
Strymonikos Gulf (N. Aegean)	Koukouras & Russo, 1991; Dounas & Koukouras, 1992;
	<b>NCMR, 2000c</b>
Thermaikos gulf (N. Aegean)	Koukouras & Russo, 1991; NCMR, 1996
Turkey (Aegean coast)	Cinar et al., 1988

the sediment is azoic (all groups zero). Numeric values between 2 and zero are nonexistent in the scale because if GI is zero the Bentix index is 2. A classification system of soft bottom macrozoobenthic communities is proposed based on the Bentix index and including five levels of ecological quality (table 3). The boundaries between classes were set keeping equal distances among classes limited only by the two extremes of the scale (2-6) and

were tested using data from various sites with known environmental pressures.

The Bentix index applies to all kind of marine soft bottom benthic data. A refinement of the upper limits of the scale is required in the case only of a type of habitat which is considered as physically stressed as is the habitat of muds. In this case the nature of the substratum with high percentage of fine particles favours the accumulation of organic

<b>Pollution</b>	<b>BENTIX</b>	<b>Ecological Quality</b>
<b>Classification</b>		Status (ECoO)
Normal/Pristine	$4.5 \leq \text{BENTIX} < 6.0$	High
Slightly polluted, transitional	$3.5 \leq$ BENTIX < 4.5	Good
Moderately polluted	$2.5 \leq$ BENTIX $< 3.5$	Moderate
Heavily polluted	$2.0 \leq$ BENTIX $< 2.5$	Poor
Azoic		Bad

**Table 3 Classification scheme of soft bottom benthic habitats based on the Bentix index.**

matter. In addition the circulation regime in muddy very sheltered bays and the morphological conditions naturally favour the accumulation of nutrients and the stratification of the water column. Thus the benthic fauna is normally dominated by some tolerant species, typical of mud (scored with 2) reducing the Bentix index even if the conditions are undisturbed by human activities.

A possible refinement of the limits of the Bentix scale for use in the muddy habitats would change the moderate class range from 2.5-3.5 to 2.5-3, the good quality class from 3.5- 4.5 to 3-4 and the high quality class from 4.5-6 to over 4. This suggestion was based on tests using benthic data from coastal muddy habitats with known environmental pressures.

# *d) Use of biological indices 1. the species richness (S)*

This index is sample size and habitat type depended. Hence, when used for classification purposes it should refer to a standard sampling area ex. 0.1m2 proposed for soft bottom coastal communities. Also the use of this index in classification requires definition of ranges of variation of S for each of the five ecological quality classes and for the various habitat types.

# *2. the index of Shannon-Wiener diversity (H')*

This index also depends on sample size and on habitat type and equally should refer to a standard sampling surface. Ranges of variation of H' corresponding to five ecological quality classes should be defined for the various habitat types accordingly. Values presented in this work are mean values from a number of replicates within a site and from a number of sites within the same geographic area and habitat type.

*e) A total of 26 data sets* (Table 2) covering 32 geographic areas within Greece and the Mediterranean (Algeria, Lyon gulf, Turkish Aegean coasts) are used in order to test and validate the methods proposed. The data originate from published documents, NCMR technical reports and/or unpublished data in the possession of the authors, communicated data from colleagues all over the Mediterranean etc. The data was used and run in its analytical format referring to the standard sample size unit.

# **Results**

# *The Bentix Index*

# *a) Application of the Bentix index to a range of data from Greek marine ecosystems*

Figure 2 shows the relative contribution of the three ecological groups in a degradation model from lowest (Bentix=0, azoic sediment) to highest ecological status (Bentix=6). The area of the diagram defined between the Bentix index values 0 and 2 is actually a non-value part of the scale as explained in the methodology.

The stations grouped for this model represent different benthic habitat types all over the Greek Seas. As it is evident communities belonging to the poor quality



*Fig. 2:* Degradation model of benthic communities health illustrating percentages of ecological groups and BENTIX index values.

status are structured with GI representing less than 13% of the fauna, GII over 30% and GIII over 50% reaching 100%. For moderate quality status GI species represent less than 30%, GII over 13% and up to 88% and GII may reach 75% of the fauna. In the good quality status the contribution of GI sensitive species rises to over 38%, GII is between 37 and 57% and GII is less than 15%. Finally high quality status is characterised by GI over 68%, GII around 30% and GIII contributes less than 4%.

#### *b) Validation of the method*

To validate the new tool of Bentix index a Greek area was chosen that presents various types of habitats and levels of quality status: Saronikos Gulf.

Saronikos gulf receives the pressures of organic pollution mainly originating from the Psittalia central outfall of Athens metropolitan area (NCMR, 2001a). The area, (including the innermost very enclosed Elefsis Bay) presents all levels of water quality and three types of habitats, namely muddy sand, sandy mud and mud. In December 2000, 24 stations were sampled using a box corer with 0.1m2 sampling surface. The stations are located in Saronikos gulf with increasing distance from the site of station S7 in Psittalia where there is the outfalls of the sewage primary treatment plant (Fig. 3). Table 4 shows the values of the biological indices, namely the community diversity (H'), the evenness of distribution J (PIELOU, 1969), the species richness (S), the Bentix index and

the derived EcoQ level in the three types of habitats.

To validate the Bentix index some water quality parameters as the Particulate organic Carbon concentration in seawater (POC), the Dissolved Oxygen concentration near the bottom (DO) and the percentage Organic Carbon content of the sediment (OC), were plotted against the Bentix index in the sampled stations (Fig. 4). The values of the abiotic parameters used in the scatter diagramms are the mean annual values of the year 2000 (table 4).

As seen in Figure 4a the index increases with Dissolved Oxygen increasing and the DO reaches the maximum value of 4.97 ml/l corresponding to the maximum value of the Bentix (4.27) and high EcoQ. The Spearman rank correlation between DO and the Bentix is statistically significant  $(p<0.01)$ . The Particulate Organic carbon concentration in seawater on the other hand shows a decrease with the increase of the index (Fig. 4b) but this negative relationship is not statistically significant. The concentration of the Organic Carbon in the sediment (OC) shows a similar pattern with POC (negative relationship with the Bentix index, although again not statistically significant) reaching the lowest value of 0.35% in the highest EcoQ station with the Bentix index reaching the highest value of 4.95 (Fig. 4c). In conclusion, the water and sediment quality attributes are directly reflected to the Bentix index.



*Fig. 3:* Map of Saronikos Gulf with stations sampled.

# *The diversity indices*

#### *a) Species richness (S).*

The species richness (S), can be a reliable tool for measuring environmental stress, provided that it is used under the following conditions:

- i. Occurring within a well defined sampling unit (standard 0.1m2): a cumulative increase of species number with sampling effort is a well-established pattern exhibited in many geographic areas and also documented in the Greek Seas. Table 5 shows that the maximum number of species (mean value) in pristine areas ranges from 88.5 to 187 depending on the surface area sampled.
- ii. From samples collected with the same gear (standard grab 0.1m2, mesh sieve 0.5mm): Results have shown that up to 40% more species (25% of an average) can be encountered when using a finer mesh sieve.
- iii. Referring to the same habitat type (ranges to be defined per habitat type): based on

data collected over a variety of soft bottom habitats in Greek waters it appears that number of species, ranges between 3.3 and 124 species per 0.1m2, depending on depth and type of substratum (Table 5). It is obvious that the communities richer in species number are those in sandy muds and muddy sands.

iv. The species identification is being done at the same taxonomic level (4 major groups or all groups).

Given all the above assumptions, an example is cited (figure 5) to demonstrate how species richness can be used to assess the ecological status of an area. The example is based on real data from an area where at least 4 classes were evidenced (S. Evvoikos Gulf following an oil spill; source: NCMR, 2001c).

# *b) Community diversity (H')*

The number of species and their relative abundance can be combined into an index that shows a closer relation to other properties of



*Fig. 4:* Relationship of the Bentix Index to water quality factors: Dissolved Oxygen concentration in the bottom layer (DO ml/l) (a), Particulate Organic Carbon concentration in seawater (POC µmol/l) (b) and Total Organic Carbon content in sediment  $(OC\%)$  (c).

the community and environment than would species richness alone. The Shannon-Wiener diversity index, developed from the information theory, is perhaps the most popular diversity index with marine biologists, has been tested in various environments and since it is calculated solely from the relative abundance of species is considered a perfectly valid parameter from a biological point of view (HEIP, 1980). However, this index is relatively biased towards the species richness aspect of diversity compared to other indices (PLATT *et al.,* 1984) and also 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. A decreasing trend with depth has been







*Fig. 5:* Trends in species richness (S) in the 5 ecological classes in shallow muddy sands related to an oil spill in S. Evvoikos Gulf.

**Table 5 Range of species variety (S) and community diversity (H') for different sampling units and habitat types.**

<b>Sampling surface</b>	S min	$H'$ min	<b>Polluted</b>	S max	$H'$ max	Reference
0.05m <sup>2</sup>	5.3	0.78	coarse sands	88.5	5.79	Coarse sands
0.1 <sup>m2</sup>	3.3	0.78	deltaic	124	6.06	Coarse sands
0.2 <sup>m2</sup>	11.8	1.21	sandy muds	126	6.30	Muddy sands
0.5 <sup>m2</sup>	28	1.82	fine sands	187	6.68	Muddy sands

established in a N. Aegean transect with minor variation of the community diversity among replicate samples (Fig. 6).

Community diversity in Greek waters has been calculated to range between 1.82 to 6.68, if calculated on pooled data. However, if calculated on a standard sampling unit (0.1m2) the maximal value is  $6.06$  bits  $\cdot$  unit<sup>-1</sup> (Table 5). Table 6 presents the range of values of H' estimated per habitat type in different areas all over Greece and some from the eastern and western Mediterranean.

As seen in Table 6, community diversity values are lower in transitional waters (lagoons, deltas, midlittoral zone) and peak in coarser sediments (mixed sands, coarse sands, coralligenous bottoms). Certainly community diversity is lowered by severe pollution stress compared with control areas or years. The maximum values of H' coincide with the pristine areas of Petalioi Gulf (Aegean), Strymonikos Gulf (coarse sands community), Milos island and Ionian Sea.

Within the same habitat type, benthic community diversity is directly related to the ecosystem quality. The distinction into five ecological classes (as defined in the WFD) is presented for one habitat type, that of the shallow muddy sands or muddy sands in protected areas (SVMC community) (Fig. 7). Values depend partly on the studied area (sampling surface), yet the same trend is



*Fig. 6:* Trend of H' according to depth calculated for each one of three replicate samples (a,b,c) in a N. Aegean transect.

**Table 6 Range of Community diversity (H') according to sampler (0.05; 0.1; 0.2; 0.5 m2) and habitat type.**

<b>Habitat type</b>	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)	$3.74/0.2m2$ (Strymonikos)
Lagoons	$0.78/0.1m2$ (Logarou)	$3.29/0.1m2$ (Papas)
Muddy sands	$3.5/0.1m2$ (Saronikos, Izmir)	$5.67/0.1m2$ (Petalioi)
		$6.68/0.5m2$ (Itea)
Muddy sands with phytal cover	$3.5/0.1m2$ (Turkey)	$5.21/0.1m2$ (Ionian)
		5.95/0.2m <sup>2</sup> (Antikyra)
Sandy muds	$1.99/0.1m2$ (Saronikos)	$4.94/0.1m2$ (Pagassitikos)
		$5.42/0.2m2$ (Pagassitikos)
Shallow muds	$1.21/0.2m2$ (Algeria)	4.35/0.2m <sup>2</sup> (Thermaikos)
	$3.17/0.1m2$ (Maliakos)	4.97/0.1m <sup>2</sup> (Strymonikos)
Deeper muds	$2.36/0.1m2$ (N. Evvoikos)	$4.04/0.1m2$ (S. Evvoikos)
<b>Shallow Sands</b>	$1.82/0.5m2$ (Marseille)	5.16/0.5 (Milos isl./Kyclades)
Deeper Sands with detritus	$2.87/0.1m2$ (Ionian)	$5.22/0.1m2$ (Ionian)
Deeper Coarse sands	$3.74/0.1m2$ (Ionian)	$6.06/0.1m2$ (Strymonikos)
Shallow muddy sands	$2.35/0.05m2$ (Geras)	$5.23/0.05m2$ (Oropos)
Coralligenous	$4.84/0.1m2$ (Chalkis)	$5.16/0.1m2$ (Ionian)
		$6.20/0.2m^2$ (Ionian)

evident along the pollution gradient, which is *Comparison of the methods* irrespective of geographic area.

A similar range of values has been calculated in muddy/sandy substrata encountered in closed gulfs. Thus, a somewhat arbitrary assessment of ecological quality status, based on long experience of the authors in closed gulfs (Saronikos, Thermaikos), can be drawn using the diversity index (Table 7). This is further supported by literature in other Mediterranean areas and refers to closed ecosystems, and to estimated values as means per 0.1m2.

# *a. Application of the methods in Saronikos gulf*

In order to cross check effectiveness of the Bentix index against or in combination with the other tools of classification (biological indices of community diversity and species richness) to assessing EcoQ, the data of the Saronikos gulf case study were used (table 4). In the diagrams of Figure 8 the community diversity and the species richness of the stations



*Fig. 7:* Trends in community diversity (H') in the 5 ecological classes in shallow muddy sands related to an oil spill in S. Evvoikos Gulf.

**Table 7 Classification scheme of muddy/sandy benthic habitats based on diversity index (H').**

bad:	$0 < H' \le 1.5$ :	azoic to very highly polluted, ex. Elefsis Bay, Thessaloniki Bay
poor:	$1.5 < H' \leq 3$ :	highly polluted, ex. Saronikos, Thermaikos
moderate:	$3 < H' \leq 4$ :	moderately polluted
good:	$4 < H' \leq 5$ :	for transitional zones
high:	$H' > 5$ :	reference sites

studied are plotted in combination with the Bentix index showing also the EcoQ according to the Bentix in increasing order. It is apparent that the community diversity is lower in the muddier sediments compared with the same quality level coarser sediments (muddy sand, sandy muds). The same is also valid for the species richness. Generally the biological indices values and especially those of the diversity index are relevant with the values of the Bentix index having lower values in the lower level quality status.

However there appear some differences in the results of the two methods, ie. stations S26B and S16B (Table 4) are classified according to Bentix to the good EcoQ, while the diversity values of 5.46 and 5.52 respectively ranks them to the high EcoQ. This is explained by the fact that H' encompasses both species richness and evenness and may be high due to increased species richness  $(S=71 \text{ in } S26B \text{ and } S=78 \$ S16B) while at the same time some tolerant species reach fairly high densities i.e. *Eunice vittata* reaches densities of 7.34% and 11.8%. This situation may be evident in the reduction of the evenness index  $(J=0.88 \text{ in } S16B)$  and is often encountered in intermediate levels of disturbance showing sometimes elevated species richness. From this example it is evident that the Bentix reflects better the EcoQ than the diversity index does, as the change in the species composition is better expressed.



*Fig. 8:* Community diversity, species richness and BENTIX index trends in Saronikos gulf.

# *b) Application of the methods in a Western Mediterranean area*

To apply the Bentix index in the Western Mediterranean and compare it with the results of the diversity indices, a set of data (MASSÉ, 1971) coming from the Bay of Prado (Gulf of Lion) was used. These macrozoobenthic data was collected from the infralittoral well sorted fine sands or shallow sands habitat. The values of the Bentix index, the biological indices H, J and S, and the corresponding EcoQ in the two stations studied are given in table 8. Station 1 is mentioned as most exposed, and more pure (greater self purification capacity) than station 2, which is more exposed to the river "Huveaune" outputs representing a source of pollution from urban and industrial wastes. It

is evident that the Bentix index values differentiate among stations reflecting the lower EcoQ of station 2, while the diversity indices values give comparable results among stations.

#### **Discussion**

Although it is not yet well established it seems that specific types of habitats or ecosystems are common throughout the Mediterranean ecoregion and that the corresponding communities can be classified into types which are similar in their basic structure and composition throughout the Mediterranean. Of course there are species that present local patterns of distribution within





the Mediterranean Sea, but in principle their congeneric or others of similar ecological significance occur in similar habitats.

Therefore a single system of benthic habitat types may be adopted for the Mediterranean and used for typology testing. In fact the typology of a given water body as defined by the WFD will be based on physical variables, but the biology ex. the description of the habitat type by using benthic indicator species, will serve as a testing and justification of the **typological** definition. A core set of species characterising each type of habitat may be derived including species presenting wide or limited distribution patterns. Similarly a group of species indicating disturbance or pollution pertaining to the Mediterranean Sea may be assembled to use in **classification** methods.

The need of the interpretation of the macrobenthic data and its use in detecting anthropogenic stress, disturbance and change has led to the development of an extensive number of concepts and numerical techniques: diversity indices, multivariate tools, graphical representations, indicator species, biotic indices (ELLIOTT, 1993; 1994).

Among them, **diversity indices** are basically an approach to biological quality through the structure of the community. The Shannon-Wiener index of diversity is, without doubt, one of most commonly used diversity indices in the assessment of pollution in marine

benthic communities. However, the use and interpretation of this and other indicators (i.e. Hill numbers, Simpson, number of species), has been subjected to much debate (CLARKE & WARWICK, 1994; JENNINGS & REYNOLDS, 2000). The values of all these indices are influenced by sampling methodology, sample size and identification procedures. Their validity with hard substrata communities is further argued because colonial organisms are not easily enumerated. Consequently, species richness and community diversity values can only be compared if the same sampling methodology has been followed, including same level of taxonomic expertise. Also these indices are habitat type dependent, which means that different ranges of values or classification schemes should apply for different habitat types.

**Graphical representation** methods of the community structure are also widely used in ecological assessment of benthic ecosystems. The log-normal distribution method (GRAY, 1981b) compares species abundance patterns with theoretical models. Another graphical representation method, the abundance/ biomass comparison (ABC) plots is commonly used in coastal waters and to a lesser degree in estuarine waters (WARWICK, 1986).

**Multivariate techniques** unlike diversity measures take into account changes in taxa and base their comparisons on the extent to which different data sets share particular species, at comparable levels of abundance. Multivariate statistics have also been used at higher taxonomic levels (genus, family and phylum) like the Phylum-level meta-analysis of WARWICK & CLARKE 1993).

An EcoQ assessment tool proposed recently is the relative abundance of **indicator species** (fragile and/or opportunistic) in respect to the total fauna, which however requires the definition of reference levels for each habitat type and the definition of the ranges of variation for each quality class to use for classification purposes (De BOER *et al.,* 2001; ZENETOS & SIMBOURA, 2001).

**Biotic indices** approach ecological quality through the use of the indicator organism concept and like multivariate methods they take into account changes in taxa. Although taxonomy may vary widely, the methodology behind establishing biotic indices may be universal.

Biotic indices based on some families of a given group, for example the Cirratulids & Paraonids (Polychaeta) have been used to assess organic pollution (BELLAN *et al.,* 1981). Also the ratio of Nematodes/Copepods has been proposed as an index for organic pollution assessment (RAFFAELLI & MASON, 1981) but has been much debated and have been not used very widely.

BELLAN (1980) proposed the ratio of the dominance of tolerant to pollution polychaete species/the dominance of the polychaete species indicative of purity as an Index of Pollution (IP) directly correlated to the degree of organic pollution. In the same philosophy is the index of r/k (r-strategy species/k-strategy species) proposed by De BOER *et al.* (2001) which considers all benthic taxa. However they emphasise the difficulty of the exact scoring of each species through the biological trait analysis.

The Infaunal Trophic Index (WORD, 1979) was designed for use in coastal waters with organic contamination only, and involves allocating species into one of four feeding groups combining the percentages in a formula. But this approach has the difficulty of determining in detail the trophic habits of many species which often is not an easy task and information is scarce on that topic.

The Biological Quality Index (BQI) developed by WILSON *et al.*(1987) is used in conjunction with the Pollution Load Index (PLI) for the characterisation of the ecological quality of estuarine ecosystems. The BQI is based on the characterisation of each intertidal zone as one of three categories (abiotic, opportunistic, stable) with the use of indicator species. The areas assigned to each category are accurately mapped and then expressed as

proportions of the total area of the estuary. The main difference with the Bentix is that the BQI is based on a qualitative estimation of the dominance of some indicator species, while the Bentix takes into account the percentages of all benthic species scored appropriately.

Another Biotic index, the Coefficient of Pollution (CoP) proposed by SATSMADJIS (1982) is based on the empirical relationships between the numbers of individuals and species in unpolluted macrobenthic communities with sediment granulometry and water depth. However, its sensitivity was doubted and its widespread application is questionable (WARWICK, 1993).

Taxonomic distinctness  $(\Delta^+)$  is a univariate (bio) diversity index. It utilises simple species lists (presence/absence data) to derive  $\Delta^+$ which encompasses not only distribution of abundance's amongst species but also the taxonomic relatedness of the species (WARWICK & CLARKE, 2001). Taxonomic distinctness is reduced in respect to increasing environmental stress and this response of the community lies at the base of this index's concept.

The concept of a biotic index based on the entire macrozoobenthos and using a formula which gives a series of continuous values has been only recently developed, at least at European scale. The first attempt to construct a single formula to obtain a continuous index was that of BORJA *et al.* (2000). ROBERTS *et al.*(1998) have also proposed an index based on macrofaunal species scored according to the ratio of each species abundance in control versus impacted samples. This index however is semi-quantitative and is site and pollution type specific.

The main difference of the formula suggested in this work for deriving BENTIX, from the formula of BORJA *et al.*(2000) is that the utilised ecological groups are eliminated from five to three giving the same weight (2) for the tolerant-second order opportunistic and the first-order opportunistic species. In this way the treated groups are posteriorly reduced to two: the sensitive and the tolerant. Also each ecological subgroup is weighted equally in relation to the others, resulting eventually to the ratio of 3 to 1 for the sensitive versus the tolerant group. As was proved from the testing and validation of the method, the reduction of the number of ecological groups involved does not affect the discriminative power of the index. It seems that a weighted ratio expressing the differential weight between the two ecological groups, describes well the shifts in the community composition balance and is effective in discriminating among five classes of ecological quality.

Reducing the number of groups has the advantage of avoiding uncertainty regarding the grouping (two groups instead of five) and also of increasing the simplicity of its calculation. Although two groups are eventually used in the calculation of the Bentix index, it is recommended to obtain the relative contribution of the three ecological groups in the fauna as it is essential for the understanding of the community composition.

At this point it should be mentioned that the Bentix index bears great similarity in its concept with the IP index of BELLAN (1980) as the latest also uses only two ecological groups. However, the main difference lies in the weighted factors used in the Bentix index formula which express the differential weight among the two ecological groups and is believed to give the Bentix index a higher descriptive value compared with the IP.

Another difference with the index of BORJA *et al.* (2000) is that the scale of classification is reversed in an ascending way from bad (0) to normal (6) so as to reflect the high EcoQ.

As tested with data coming from other parts of the Mediterranean as the Prado bay (gulf of Lion) (MASSÉ, 1971) the Bentix index has global validity, at least within the Mediterranean Sea. Indeed, the Bentix index method applied in the case of the Prado gulf data differentiated between stations and reflected well the ecological quality status of these areas as described by the environmental characteristics and the quantitative analysis of the macrofaunal populations (MASSÉ, 1971).

On the other hand the indices of diversity, evenness and species richness did not show great differences between the two stations. This is attributed to the fact that the area is generally highly populated with benthic fauna and present some especially high densities of some species. These extremely abundant species which lower the evenness and diversity indices at both stations almost equally, include in the case of station 1 some sensitive species like *Spiophanes bombyx* and the Turbellaria worms, while in station 2 they are mostly opportunistic species as *Phoronis psammophila* and *Spio decoratus.*

In an attempt to evaluate and compare the indices discussed, two properties of the indices were separately valued: dependence on external factors and the ability of the indices to reflect the different community attributes. The overall dependence of an index on external factors such as the sample size, the habitat type and the degree of taxonomic effort required to obtain the index, represents the "robustness" of the index. An index which is independent from the sample size, applies to a great range of habitat types and does not require exhaustive taxonomic effort, is a robust index with high practical value. On the other hand, an index should reflect and describe the structural and biotic attributes of a community which relate to the response of the benthic communities to disturbance factors. Such communities attributes are for example the species richness, the evenness of the species' distribution and the faunal composition in relation with sensitivity or resistance of the species to disturbance factors. The ability of an index to integrate these attributes corresponds with the power of the index to discriminate among different ecological quality classes and represents the "effectiveness" of the index. Table 9 gives an overall qualitative evaluation of the indices discussed in this work, viewing

		Dependence on external factors				Reflection of community attributes			
Index	Habitat Type	Sample size	Taxon. effort	Robustness	S	Evenness	<b>Species</b> sensitivity	Effectiveness	
Bentix	No	N <sub>0</sub>	Moderate	Very good	N <sub>0</sub>	N <sub>0</sub>	Yes	Good	
H <sup>'</sup>	Moderate	Moderate	Yes	Moderate	Yes	Yes	N <sub>0</sub>	Good	
$\overline{s}$	Yes	Yes	Yes	Moderate	Yes	No	N <sub>0</sub>	Moderate	

**Table 9 Evaluation of the indices.**

them as tools for the classification of benthic communities into ecological quality classes.

As seen in Table 9 the Bentix index proposed is a very robust and adequately effective tool in classifying benthic communities into ecological quality classes. Its robustness lies in the fact that it is largely habitat type and sample size independent and thus has a potential for global application. Its effectiveness in discriminating between ecological classes is based on its ability to reflect the faunal composition in relation with the resistance of species to disturbance factors.

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

The biological index of H' gives a good description of the community structural and biotic aspects. However, only if used in combination with the Species richness, the evenness index, and maybe the faunistic data, so as to highlight the dominance of opportunistic species, gives a safe description of the community structure and composition. Besides, its high dependence on external factors renders the index inconvenient for use in a benthic community ecological classification scheme.

#### **Acknowledgements**

We would like to thank the anonymous reviewers for their contribution in the improvement of the paper and the Chief Editor Dr. E. Papathanassiou for motivating us to produce this work.

**ANNEX I: Type of communities, scoring of indicator species, and examples of occurrence with percentage abundance.** 

<b>Muds</b>			
<b>CODE</b>	<b>SPECIES</b>	EG	<b>REGION</b>
			(percentage abundance %)
Pol	Ampharete acutifrons	$\overline{2}$	
Pol	Aricidea claudiae	$\mathbf{1}$	Nestos $(4.9)$
Pol	Chaetozone setosa	3	Kavala Gulf, Strymonikos (4.7)
Pol	Cossura coasta	$\overline{2}$	Strymonikos (4.1)
	Goniada maculata	$\mathbf{1}$	
Pol	Hydroides elegans	3	Amvrakikos (39.6)
Pol	Laonice cirrata	$\mathbf{1}$	Atalanti Bay, Amvrakikos
Pol	Lepidasthenia maculata	$\mathbf{1}$	France
Pol	Levinsenia gracilis	$\overline{c}$	Strymonikos (7.8), Gulf of Thessaloniki (3.6)
Pol	Lumbrineris latreilli	$\overline{2}$	Atalanti (38), Kavala gulf, Strymonikos (11),
			Nestos (16,7), Gulf of Thessaloniki (2.7)
Pol	Maldane glebifex	$\mathfrak{2}$	
Pol	Maldane sarsi	$\overline{2}$	
Pol	Marphysa bellii	$\mathbf{1}$	
Pol	Metasychis gotoi	$\mathbf{1}$	
Pol	Monticellina dorsobranchialis	$\overline{c}$	Catalan coasts, Strymonikos (14.7)
Pol	Nephtys hystricis	$\mathbf{1}$	Atalanti, S. Evvoikos, Maliakos (9.5),
			Strymonikos (5.3)
Pol	Ninoe armoricana	$\mathbf{1}$	S. Evvoikos, Strymonikos
Pol	Paradiopatra calliopae	$\mathbf{1}$	Gulf of Thessaloniki (7.8)
Pol	Pectinaria belgicae	1	
Pol	Praxillella gracilis	$\mathbf{1}$	Strymonikos
	Prionospio ehlersi	$\mathbf{1}$	Kerkira (4.4)
Pol	Sternaspis scutata	$\mathfrak{2}$	Atalanti (8), S. Evvoikos, Amvrakikos,
			Maliakos (20), Pagassitikos (5), Strymonikos (6.4)
			Gulf of Thessaloniki (28)
Pol	Sthenolepis yhleni	$\mathbf{1}$	Strymonikos
Pol	Terebellides stroemi	$\mathbf{1}$	Strymonikos
Mol	Abra nitida	$\overline{2}$	Atalanti (1), Strymonikos (4.7)
Mol	Acanthocardia paucicostata	$\mathbf{1}$	
Mol	Corbula gibba	3	Amvrakikos (84.2)
Mol	Cultellus adriaticus	$\mathbf{1}$	Atalanti
Mol	Hyala vitrea	$\sqrt{2}$	Nestos $(7.6)$
Mol	Mysella bidentata	$\mathfrak{2}$	Atalanti (7), Amvrakikos, Maliakos (32),
			strymonikos $(5.7)$
Mol	Nucula sulcata	$\mathbf{1}$	Catalan coasts
Mol	Nucula tenuis	$\mathbf{1}$	Strymonikos
Mol	Nucula turgida	$\mathbf{1}$	Nestos (12.2)
Mol	Thyasira alleni	$\overline{2}$	Strymonikos (7.1)
Mol	Thyasira flexuosa	3	La Coruna (Iberian Atlantic coasts), Maliakos (9.5),
			Nestos (7.6), Thermaikos (4.8)
Mol	Timoclea ovata (Banyuls)	$\overline{c}$	Banyuls bay (French Medit.)
Mol	Turritella communis	$\mathbf{1}$	Kavala gulf, Izmir Bay, Adriatic, Strymonikos (4.7),
			Gulf of Thessaloniki (3)
Cru	Ampelisca typica		Nestos $(7.4)$
Cru	Callianassa subterranea	$\mathbf{2}$	S. Evvoikos







































#### **ANNEX II:**

#### **List of tolerant species with scoring number, examples of occurrence (with percentage abundance) and ecological traits.**  $\overline{\phantom{0}}$



'n







#### **References**

- ALBERTELLI, G. & FRASCHETI, S., 1995. A quantitative study of a macrobenthic community in the Ligurian Sea (North-Western Mediterranean). *Oebalia,* 11: 103-113.
- BAKALEM, A., 1981. Le peuplement des sables fins à *Ophiura texturata-Donax semistriatus* de la baie d'Alger: évolution dans le temps *Rapport de la Comission internationale pour l' exploration Scientifique de la Mer Méditerranée*, 27:2.
- BAKALEM, A., 2001. Diversité de la macrofaune des sables fins de la côte Algerienne. *Rapport de la Comission internationale pour l' exploration Scientifique de la Mer Méditerranée,* 36: 355.
- BELLAN, G., 1980. Annelides Polychetes des substrats solides de trois milieux pollues sur les cotes de Provence. (France): Cortiou, Golfe de Fos, Vieux Port de Marseille. *Tethys* 9(3): 260-278.
- BELLAN, G., JORAJURIA-OLIVARI, A. & PICARD, J., 1981. Le peuplement des substrats meubles dans le couloir d'écoulement des eaux

usées de la Ville de Marseille. *Vemes Journees Etud., Pollutions, Cagliari, 1980. CIESM., Monaco:* 649-656.

- BELLAN-SANTINI, D., 1980. Relationship between populations of Amphipods and pollution. *Marine Pollution Bulletin,* 11: 224-227.
- BIANCHI, C.N., CEPPODOMO, I., GALLI, C. & SGORBINI S., 1993. Benthos dei Mari Toscani. I: Livorno-Isola d' Elba (Crociera ENE 1985). In: *Archipelago Toscano* by Ferretti *et al.,*ENEA, 263- 290.
- BORJA, A., FRANCO, J. & PEREZ, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin,* 40(12): 1100-1114.
- CARDELL, M.J., SARDA, R. & ROMERO, J., 1999. Spatial changes in sublittoral soft-bottom polychaete assemblages due to river inputs and sewage discharges. *Acta Oceanologica,* 20(4): 343- 351.
- CINAR, M.E., ERGEN, Z., OZTURK, B. & KIRKIM, F., 1988. Seasonal analysis of zoobenthos associated with a *Zostera marina* L., bed in Gulbanche Bay (Aegean Sea, Turkey). P.S.Z.N.I.: *Marine Ecology,* 19(2): 147-162.
- CINAR, M.E., ERGEN, Z. KOKATAS, A.& KATAGAN., T., 2001. Zoobenthos of the probable dumping area in Izmir Bay (Aegean Sea). *Rapport de la Comission internationale pour l' exploration Scientifique de la Mer Méditerranée,* 36: 374.
- CLARKE, K.R. & WARWICK, R.M., 1994. Change in marine communities: an approach to statistical analysis and interpretation. Plymouth Marine Laboratory, Plymouth: 144p.
- CORBERA, J. & CARDELL, M.J., 1995. Cumaceans as indicators of eutrophication on soft bottoms. *Sciencia Marina,* 59 (Supl.1): 63-69.
- DE BOER, W.F., DANIELS, P. & ESSINK, K., 2001. Towards Ecological Quality Objectives for North Sea Benthic Communities. National Institute for Coastal and Marine Management (RIKZ), Haren, the Netherlands. Contract RKZ 808, Report nr 2001-11, 64 p.
- DESBRUYERES, D., GUILLE, A. & RAMOS, J.M., 1972-73. Bionomie benthique du plateau continental de la côte catalane espagnole. *Vie Milieu,* 23(2), 335-363.
- DOUNAS, C.G., & KOUKOURAS, A.S., 1992. Circalittoral macrobenthic assemblages of Strymonikos Gulf (North Aegean Sea). P.S.Z.N.I.: *Marine Ecology,* 13(2), 85-99.
- EEC, 2000. Directive of the European parliament and of the Council 2000/60/EC establishing a framework for community action in the field of Water Policy. PE-CONS 3639/1/00.
- ELLIOTT, M., 1993. Recent developments in marine macrobenthic community analysis. *Proceedings of the 4th National Symposium on Oceanography and Fisheries, Rhodes (isl)., Greece), 26-29 April,* pp. 144-155.
- ELLIOTT, M., 1994. The analysis of macrobenthic community data. *Marine Pollution Bulletin,* 28(62- 64).
- ELLIOTT, M., 1996. The derivation and values of ecological quality standards and objectives. *Marine Pollution Bulletin,* 32(11) : 762-763.
- GLEMAREC, M., 1986. Ecological impact of an oilspill: utilisation of biological indicators. IAWPRC-

NERC Conference, July 1985. *IAWPRC Journal* 18: 203-211.

- GOUVIS, N., KEVREKIDIS T. & KOUKOURAS, A., 1997. Temporal changes of a macrobenthic assemblage in Evros Delta (Noth Aegean Sea). *Internationale Revue der Gesamten Hydrobiologie.,* 82(1): 67-80.
- GRALL, J. & GLEMAREC, M., 1997. Using biotic indices to estimate macrobenthic community perturbations in the Bay of Brest. *Estuarine and Coastal Shelf Science,* 44A: 43-53.
- GRAY, J.S., 1979. Pollution-induced changes in populations.*Philosophycal Transactions of the Royal Society of London.* Series B, 286: 545-561.
- GRAY, J.S. , 1981(a). The ecology of marine sediments: an introduction to the structure and function of benthic communities. Cambridge University Press, Cambridge.
- GRAY, J.S., 1981(b). Detecting pollution-induced changes in communities using the log-normal distribution of individuals among species. *Marine pollution Bulletin,* 12: 173-176.
- GREMARE, A., AMOUROUX, J.M. & VETION, G., 1998. Long-term comparison of macrobenthos within the soft bottoms of the Bay of Banyuls-surmer (northwestern Mediterranean Sea). *Journal of Sea Research,* 40: 281-302.
- GRIMES, S. & GUERAINI, C., 2001. Etat de reference de la macrofaune benthique du port de Djendjen (Algerie orientale). *Rapport de la Comission internationale pour l' exploration Scientifique de la Mer Mediterranee,* 36: 388.
- HEIP, C., 1980. Meiobenthos as a tool in the assessment of marine environmental quality. *Rapports et Proces-Verbaux des Reunions (Monaco),* 179: 182-187.
- HILY, C., 1984. Variabilité de la macrofaune benthique dans les milieux hypertrophiques de la Rade de Brest. Thèse de Doctorat d' Etat, Univ. Bretagne Occidentale. Vol.1., 359 p; Vol 2.,337 p.
- IVESA, L., ZAVODNIK N. & JAKLIN, A., 2001. Benthos of the *Caulerpa taxifolia* settlement at Malinska (Croatia, Adriatic Sea). *Rapport de la Comission internationale pour l' exploration Scientifique de la Mer Méditerranée*, 36: 393.
- JENNINGS, S. & REYNOLDS, J.D., 2000. Impacts of fishing on diversity: from pattern to process. In: *Effects of fishing on non-target species and habitats,* by MJ. Kaiser & S.J. de Groot, Blackwell Science, Oxford: 235-250.
- KARAKASSIS, I. & ELEFTHERIOU, A., 1997. The continental shelf of Crete: structure of macrobenthic communities. *Marine Ecology Progress Series,* 160: 185-196.
- KOUKOURAS, A. & RUSSO, A., 1991. Midlittoral soft substratum macrofaunal assemblages in the North Aegean Sea. *P.S.Z.N.I.: Marine Ecology,* 12(4): 293-316.
- KOUTSOUBAS, D., DOUNAS, C., ARVANITIDIS, C., KORNILIOS, S., PETIHAKIS, G., TRIANTAFYLLOY G. &. ELEFTHERIOU, A., 2000. Macrobenthic community structure and disturbance assessment in Gialova Lagoon, Ionian Sea. *ICES Journal of Marine Science,* 57: 1472-1480.
- LAMY, N. & GUELORGET, O., 1995. Impact of intensive aquaculture on the soft substratum benthic communities in the Maditerranean lagoonal environments. *Journal de Recherche Oceanographique,* 20(1-2): 1-8.
- LOPEZ-JAMAR, E., GONZALEZ G. & MEJUTO, J., 1987. Ecology, growth and production of *Thyasira flexuosa* (Bivalvia, Lucinacea) from Ria De La Coruna, north-west Spain. *Ophelia,* 27(2): 111-126.
- MAJEED, S.A., 1987. Organic matter and biotic indices on the beaches of North Brittany. *Marine Pollution Bulletin,* 18(9) : 490-495.
- MARANO, G., PASTORELLIS, A.M., DE ZIO, V., ROSITANI L. & VACCARELLA, R., 1989. Comunita a *Chamelea gallina* (L.) nell' Adriatico pugliese. *Oebalia* 15(1), N.S.: 169-182.
- MASSE, H.L., 1971. Etude quantitative de la macrofaune de peuplements des sables fins infralittoraux: II La baie du Prado (Golfe de Marseille). *Tethys,* 3(1): 113-158.
- MASSE, H.L., 1998. Consequences à long terme de travaux d'aménagements littoraux sur la macrofaune des sables fins de deux stations de la baïe du Prado (Méditerranée Nord-Orientale-Golfe de Marseille). *Vie Milieu,* 48(2): 79-87
- MORGANA, J.G. & NAVIGLIO, L., 1995. The zoobenthic community of the Orbetello lagoon (Central Italy). *Oebalia,* XXI: 125-136.
- NCMR, 1987. Oceanographic and ecological survey of the SE Aegean Sea (1983-84). Data Report, NCMR (in Greek).
- NCMR, 1989(a). Survey of the benthic fauna in the Central Aegean Sea (Kyclades-Saronikos) A.

Zenetos (Ed.). Technical Report, 107p. (In Greek).

- NCMR, 1989(b). Oceanographical study of the Amvrakikos Gulf, 4.Biological oceanography. Ch. Tziavos (Ed.),Technical Report, p. 95. (In Greek).
- NCMR, 1990. Ecological study of Geras gulf. V.A. Catsiki (Ed.), Technical Report, 243p. (In Greek).
- NCMR, 1992. Environmental study of the Northern Evvoikos Gulf. E. Papathanasiou, (Ed.), Technical Report, 243p. (In Greek).
- NCMR, 1994. Study of the impact of bauxite residues discharge on the benthic communities of Korinthikos Gulf. A. Zenetos (ed). Technical Report, 93 p. (In Greek).
- NCMR, 1996. Study of the marine ecosystem of Thermaikos Gulf, Ch. Anagnostou (Ed.), Technical Report, 431p. (In Greek)
- NCMR, 1997. Study of the marine ecosystem of E-SE Attica from the Bay of Marathon to the Bay of Anavissos. E. Christou (Ed.), Technical Report, 377p. (In Greek).
- NCMR, 1998. Study of the environmental impact of dumping coarse metalliferous waste in the area off Larymna. N. Simboura (Ed.), Technical Report, 156 p. (In Greek).
- NCMR, 1999. Monitoring of the Saronikos Gulf ecosystem affected by the Psittalia Sea outfalls. I.Siokou-Fragou (Ed.), Technical Report, 338p.
- NCMR, 2000(a). Development of an integrated policy towards a sustainable management of Pagassitikos Gulf: pelagic-benthic ecosystemecotoxicology. Technical Report NCMR , M.A. Pancucci-Papadopoulou (Ed), (In Greek).
- NCMR, 2000(b). Monitoring of the ecosystem of the Papas lagoon, Cape of Araxos/Achaia and suggestions for the management and protection of the lagoon. Technical Report NCMR, K. Pagou (Ed), (In Greek).
- NCMR, 2000(c). The zoobenthic communities of Strymonikos Gulf and the northern coasts of the Aegean Sea. INTEREG II: Development of Infrastructure for reducing interregional pollution in the N. Aegean Sea. Technical report, D. Georgopoulos (Ed), (In Greek).
- NCMR, 2001(a). Monitoring of the Saronikos Gulf ecosystem affected by the Psittalia Sea outfalls. I.Siokou-Fragou (Ed.), Technical Report, 231p.
- NCMR, 2001(b). Study of the environmental impact of dumping coarse metalliferous waste in N.

Evvoikos gulf. N. Simboura (Ed.), Annual Technical Report, 70p. (In Greek).

- NCMR, 2001(c). Study of the short- and mid-term impact of oil pollution in South Evvoikos Gulf. I. Chadjianestis (Ed.), Technical Report, 32p. (In Greek).
- PANCUCCI-PAPADOPOULOU, M.A., 1996. The Echinodermata of Greece. *Fauna Greciae. Hellenic Zoological Society* 6, 162p.
- PAPAZACHARIAS, A., KOUKOURAS, A., KEVREKIDIS, T. & VOULTSIADOU, E., 1998. Infra- and circalittoral soft bottom substratum macrofaunal assemblages of Kavala Gulf (Aegean Sea). *Internationale Revue der Gesamten Hydrobiologie,* 83(5-6): 461-477.
- PEARSON, T.H. & ROSENBERG, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Revue,* 16:229-311.
- PERES, J.M. & BELLAN, G., 1973. Apercu sur l'influence des pollutions sur les peuplements benthiques, in: *Marine Pollution and Sea Life,* by M. Ruivo, Fishing News, W. Byfleet, Surrey: 375p.
- PERES, J.M. & PICARD, J., 1964. Nouveau manuel de bionomie benthique de la Mer Méditerranée. *Rec. Trav. Stn. mar. Endoume,* 31(47), 137 p.

PHILLIPS, D.J.H., & RAINBOW, P.S., 1994. Biomonitoring of Trace Aquatic Contaminants. Chapman & Hall, London.

- PICARD, J., 1965. Recherches qualitatives sur les biocénoses marines des substrats meubles dragables de la région marseillaise. *Rec. Trav. Stn. mar. End.,* 36(52), 1-160.
- PIELOU, E.C., 1969. The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology,* 13:131-144.
- PLATT, H.M., SHAW, K.M & LAMBSHEAD, P.J.D., 1984. Nematode species abundance and their use in the detection of environmental perturbations. *Hydrobiologia,* 118: 59-66.
- RAFFAELI, D.G. & MASON, C.F., 1981. Pollution monitoring with Meiofauna: Using the ration of Nematods-Copepods. *Marine Pollution Bulletin,* 12(5): 158-163.
- REIZOPOULOU, S., THESSALOU-LEGAKI, M. & NICOLAIDOU, A. 1996. Assessment of disturbance in Mediterranean lagoons: an evaluation of methods. *Marine Biology,* 125:189- 197.
- ROBERTS, R.D., M.G. GREGORY & B. A. FOSTERS, 1998. Developing an efficient macrofauna mointoring index from an impact study- a dredge spoil example. *Marine Pollution Bulletin,* 36(3): 231-235.
- ROS, J.D. & CARDELL, M.J., 1991. Effect on benthic communities of a major input of organic matter and other pollutants (coast off Barcelona, western Mediterranean). *Toxicol. Environ. Chem.,* 31-32: 441-450.
- SALEN-PICARD, C., 1983. Schemas d'evolution d'une biocénose macrobenthique du substrat meuble. *Comptes Rendus de l'Academie des Sciencies de Paris* 296: 587-590.
- SALEN-PICARD, BELLAN, G. BELLAN-SANTINI, D., ARLHAC, D. & MARQUET, R., 1997. Long-term changes in a benthic community of a Mediterranean gulf (Gulf of Fos). *Oceanologica Acta,* 20(1): 299-310.
- SATSMADJIS, J., 1982. Analysis of benthic data and measurement of pollution. *Revue Internationale Oceanographie Medicale,* 66-67: 103- 107.
- SHANNON, C. E. & WEAVER, W., 1963. *The Mathematical Theory of Communication.* The University of Illinois Press, Urbana, 117 pp.
- SIMBOURA, N. & A. NICOLAIDOU, 2001. The Polychaetes (Annelida, Polychaeta) of Greece: checklist, distribution and ecological characteristics. *Monographs on Marine Sciences,* Series no 4. NCMR.
- SIMBOURA, N., ZENETOS, A., THESSALOU-LEGAKI, M., PANCUCCI & A. NICOLAIDOU, M.A. 1995(a). Benthic communities of the infralittoral in the N. Sporades (Aegean sea): a variety of biotopes encountered and analysed. P.S.Z.N.I., *Marine Ecology,* 16(4): 283-306.
- SIMBOURA, N. ZENETOS, A. PANAYOTIDIS, P. & MAKRA, A., 1995(b). Changes of benthic community structure along an environmental pollution gradient. *Marine Pollution Bulletin,* 30(7): 470-474.
- SIMBOURA, N., ZENETOS, A. PANCUCCI-PAPADOPOULOU, M.A. THESSALOU-LEGAKI, M. & PAPASPYROU, S., 1998. A baseline study on benthic species distribution in two neighbouring gulfs, with and without access to bottom trawling. P.S.Z.N.I., *Marine Ecology,* 19(4):293-309.
- WARWICK, R.M., 1986. A new method for detecting pollution effects on marine macrobenthic communities. *Marine Biology,* 92: 557-562.
- WARWICK, R. M., 1993. Environmental impact studies on marine communities: Pragmatical considerations. *Australian Journal of Ecology,* 18: 63-80.
- WARWICK, R.M. & CLARKE, K.R., 1993. Comparing the severity of disturbance: a metaanalysis of marine macrobenthic community data. *Marine Ecology Progress Series,* 92: 221-231.
- WARWICK, R.M. & CLARKE, K.R., 2001. Practical measures of marine biodiversity based on relatedness of species. *Oceanography and Marine Biology: an Annual Review,* 39:207-231.
- WILSON, J.G., DUCROTOY, J.P. DESPREZ M. & ELKAIM, B., 1987. Application of two estuary quality indices to the central and western channel: status of the Somme and Seine estuaries (France). *Vie Milieu,* 37(1): 1-11.
- WORD, J. Q., 1979. The Infaunal Trophic Index. *Southern California Coastal Water Research Project Annual Report,* El Segundo, California. 19-39.
- ZAVODNIK, D., SPAN, A. ZAVODNIK, N. SIMUNOVIC A. & ANTOLIC, B., 1981. Benthos of the western coast of the island Krk (Rijeka Bay, the North Adriatic Sea). *Thalassia Jugoslavica,* 17(3/4): 285-337.
- ZENETOS, A., BEI, F. & NICOLAIDOU, A., 1991. Erratic occurrence of benthic fauna in a shallow Mediterranean area: an indirect effect of manmade disturbance. *Marine Pollution Bulletin,* 22(12): 618-622.
- ZENETOS, A., CHRISTIANIDIS, S., PANCUCCI, M.A., SIMBOURA, N. & TZIAVOS, CH., 1997. Oceanologic study of an open coastal area in the Ionian Sea with emphasis on its benthic fauna and some zoogeographical remarks. *Oceanologica Acta,* 20(2): 437-451.
- ZENETOS, A. & SIMBOURA, N., 2001. Soft bottom benthic indicators. *Rapport de la Comission internationale pour l' exploration Scientifique de la Mer Méditerranée,*(36) 339.