Mediterranean Marine Science

Vol 13, No 1 (2012)

Indicators for the Sea-floor Integrity of the Hellenic Seas under the European Marine Strategy Framework Directive: establishing the thresholds and standards for Good Environmental Status

N. SIMBOURA, A. ZENETOS, M.A. PANCUCCI-PAPADOPOULOU, S. REIZOPOULOU, N. STREFTARIS

doi: [10.12681/mms.31](https://doi.org/10.12681/mms.31)

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net

Indicators for the Sea-floor Integrity of the Hellenic Seas under the European Marine Strategy Framework Directive: establishing the thresholds and standards for Good Environmental Status

N. SIMBOURA, A. ZENETOS, M.A. PANCUCCI-PAPADOPOULOU, S. REIZOPOULOU and N. STREFTARIS

Hellenic Centre for Marine Research, Institute of Oceanography, P.O. Box 712, 19013, Anavissos, Attica, Greece

Corresponding author: msim@hcmr.gr

Received: 20 March 2012; Accepted: 20 April 2012; Published on line: 6 June 2012

Abstract

A data set of 625 samples of benthic macroinvertebrates collected from the Hellenic Seas (Ionian and Aegean) was used to establish thresholds and reference standards for two of the indicators addressing the descriptors of Sea-floor Integrity under the Marine Strategy Framework Directive (MSFD): species diversity and richness and the ratio of sensitive species to tolerant species. The dataset was categorised according to the baseline ecological status assessment of the respective water bodies under the Water Framework Directive (WFD). Species diversity and richness were characterised using the Shannon diversity and species richness indices, respectively, and were analysed for three pre-defined substrate types, three depth zones and three sample-size categories, and the significant categories were statistically validated. Good Environmental Status (GEnS) threshold and reference values were established for the valid combinations of categories denoted as 'ecotypes' through the use of a boxplot and an analysis of variance. The limitations and specifications for an overall GEnS assessment using the above indices are highlighted based on the WFD experience. For the ratio of sensitive species to tolerant species, the BENTIX index classification scale is proposed for GEnS assessment, and an integrated approach to the assessment of diversity and species richness is suggested. Finally, the regionality of the tested indices in relation to the two Mediterranean sub-regions, including the Hellenic area, was tested.

Keywords: Marine Strategy Framework Directive, Sea-floor Integrity, Hellenic Seas, Good Environmental Status, diversity, species richness.

Introduction

The Benthos Ecology Working Group (BEWG) of the International Council for the Exploration of the Sea (ICES) produced a viewpoint article addressing the ways in which the principles of the European umbrella regulations for water systems, the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD), have been applied (Van Hoey *et al.*, 2010). The development of benthic indicators and the definition of 'pristine' and/or sustainable conditions are highlighted among the principles. The selection of appropriate indicators with complementary properties and related to the Directives' objectives, including the integration of single univariate indicators, was prioritised by the BEWG. Specifically, Van Hoey *et al.* (2010) stressed the need to improve the benthic indicators already developed in the context of the WFD to assess structural and functional benthic aspects in the MSFD. There is a greater need for the incorporation of these indicators in the case of the Sea-floor Integrity Descriptor (D6) due to its more integrative and complex character.

Later, Rice *et al.* (2012) produced an elaborate paper on Sea-floor Integrity indicators based on the report of the EU Sea-floor Integrity Task Group (TG) (Rice *et al.*, 2010). In this work, the potential indicators of the attributes of Sea-floor Integrity, their features, pressures and impacts are analysed and described. The way forward is envisaged as including the selection of proper indicators at a regional scale and the setting of reference levels for selected indicators at local scale.

The European Commission reached a decision (EC, 2010) concerning the principal criteria and indicators for use in the initial assessment by member states (MS). According to this Decision for Sea-floor Integrity, two criteria were set: 'Physical damage' and 'Condition of benthic community'. The following indicators were defined for the latter criterion: the presence of particularly sensitive and/or tolerant species (6.2.1.), indices assessing species diversity and richness and the proportion of opportunistic to sensitive species (6.2.2.), the proportion of biomass or the number of individuals above a specified length/size (6.2.3.) and parameters describing the size spectrum of benthic communities (6.2.4.).

An informative work by Borja *et al.* (2010) presents a way to apply the experience gained from the WFD to the implementation of the MSFD. The work outlines the overlaps and conflicts between the two directives. Subsequently, Borja *et al.* (2011) presented a methodological approach for the assessment of environmental status in the case of the Basque country, including indicators from the WFD.

The purpose of the present paper is to propose a set of indicators and their boundary values for GEnS (Good Environmental Status) for the MSFD regional seas of the Hellenic area [the Eastern Mediterranean (Aegean Sea) and the Central Mediterranean, including the Ionian Sea]. For this purpose, the paper employs the knowledge and experience in the implementation of some of these indicators already addressed within the WFD for assessing Good Ecological Status (GES).

The BENTIX (acronym for **BEN**thic **I**nde**X**) (Simboura & Zenetos, 2002) was used for the classification of benthic macroinvertebrates in the implementation of the WFD in Hellenic coastal waters (HCMR, 2008). BENTIX is a biotic index based on the concept of indicator groups and uses the relative contribution of two general ecological groups of taxa, the tolerant and the sensitive, weighting them according to the ratio of their occurrence in the benthic fauna. The index was designed for the Mediterranean benthic ecosystem and has been tested using various anthropogenic pressures, such as eutrophication and organic pollution (Simboura *et al.,* 2005; Simboura & Reizopoulou, 2007, 2008), mining residues (Simboura *et al.*, 2007) and aquaculture (Simboura & Argyrou, 2006) in Greece, Cyprus and the Western Mediterranean (GIG, 2008, 2012).

The index has been successfully intercalibrated with other Mediterranean classification metrics through the Two-Phases Mediterranean Geographical Intercalibration Group (MED-GIG) Intercalibration (IC) exercise (GIG, 2008; 2012). These other metrics include the AZTI's Marine Biotic Index (AMBI) (Borja *et al.*, 2000), the multimetric approach M-AMBI (Borja *et al.*, 2004; Muxica *et al.*, 2007), the MEDOCC (from Mediterranéo Occidental) (Pinedo & Jordana, 2008) and the Benthic Opportunistic Polychaetes Amphipods index (BOPA) (Dauvin & Ruellet, 2007). With this procedure, the index was tested by considering its response to gradients of selected disturbance factors. These factors included the organic carbon content of the sediment and a newly developed land-use pressure index, the Land Use Simplified Index (LUSI) (Flo *et al.*, 2011).

The present work addresses the indicators of Shannon diversity and species richness and the relationship of these indicators to the proportion of sensitive to tolerant species assessed by the BENTIX index under the MSFD.

Material and Methods

A large data set, consisting of 625 benthic samples from stations throughout the Aegean and Ionian Seas and their embayments belonging to the Aegean and Levantine and Ionian-Mediterranean regional seas was used. The data were obtained from samples collected from various soft-bottom substrata and from depths ranging from 2 to 1350 m (Table 1). The data were generated from projects conducted at the Hellenic Centre for Marine Research (HCMR) and were used for the initial assessment for the WFD ecological status classification for the Hellenic coastal waters. An analysis of variance was applied to the data with STATGRAPHICS CENTURION 2009, StatPoint Technologies, Inc. software.

To test the significance and variation of factors other than disturbance in shaping the values of diversity and species richness, the depth and substratum type for each sample were categorised in pre-defined classes according to the basic principles of benthic bionomy for the Mediterranean Sea (Pérès & Picard, 1964; Bellan Santini, 1994). The sample size was not uniform for the data and was categorised according to three sample-size classes.

The BENTIX index was not included in this analysis because this metric's boundaries are intrinsically defined by a paired-metrics analysis across a pressure gradient and with weighting of indicator taxa. Moreover, BENTIX is not sample-size dependent because it is a biotic index. The BENTIX index is used for the assessment of GEnS under indicator 6.2.1 (presence of sensitive and/or tolerant species) and partly under indicator 6.2.2 (proportion of opportunistic to sensitive species) in conjunction with the other two indicators of 6.2.2, namely species diversity and richness.

These analyses served to categorise the data set (Table 2) according to four variables: Ecological Quality Status (EQS); as assessed under the implementation of WFD in Hellenic coastal waters (HCMR, 2008); sample size; substratum type and depth.

The EQS classes are defined as follows: high=1, good=2, moderate=3, poor=4 and bad=5.

The sample size was categorised as follows: category 1 for sample sizes less than 0.1 m^2 (usually 0.045 m^2 for the Ponar grab), category 2 for a standard sample size of 0.1 m^2 (for the Van Veen or Smith-McIntyre grabs) and category 3 for sample sizes greater than 0.1 m^2 (usually 0.15 or 0.2 m^2 for non-reducible aggregated samples). Almost all samples were processed through a sieve with a mesh size of 1 mm.

The substratum type was categorised as follows: category 1 for purely muddy sediments (more than 90% silt and clay); category 2 for heterogeneous mixed sediments of muddy sand or sandy mud with various admixtures of fine and coarser material and/or biogenic detritus; and category 3 for homogeneous pure sands (100% coarse material), gravel or stone without biogenic detritus.

Table 1. Areas of macroinvertebrate fauna samples, number of stations, dates of sampling and sources. **Sources cited as NCMR or HCMR refer to unpublished Helllenic Centre for Marine Research reports not included in the literature list but available in HCMR library.*

MSFD Region	No of Stations	DEPTH m	DATE	Source*
CMED (Ionian Sea)				
Amvrakikos gulf	13	12-55	1987	NCMR 1989b
Antikyra Bay	37	5-270	2002,2006,2010	NCMR 1995; HCMR 2003a,b; HCMR 2010d
Echinades isls	4	19-40	2003	HCMR 2003c
Ipeirus coasts	22	10-104	1990	NCMR 1992d; Zenetos *et al.,* 1997; Webb *et al.,* 2009
Kalamas-IHgoumenitsa	10	3-30	1999	HCMR 2000c
Kerkyra Sea	12	25-65	1991	NCMR 1992c
Laganas gulf (Zakynthos)	6	13-60	2006	HCMR, 2006
Lakonikos gulf	11	10-90	1991	NCMR, 1992b
Messiniakos gulf	16	15-50	2006,2010	HCMR 2010a
W. Peloponissos (Kefalinia)	9	24-1350	2000	HCMR 2000c
EMED (Aegean Sea)				
Cretan Sea	56	10-60	1988	Karakassis, 1991
Elefsis gulf	20	15-33	2000-2010	HCMR 2000c;2010e
Geras gulf, Lesvos isl	9	6-40	1988	NCMR 1990; Zenetos, Papathanassiou 1989; Bogdanos *et al.,* 2002 ; Webb *et al.,* 2009
Kalloni gulf, Lesvos isl	13	1-15	1988	NCMR 1996b
Kavala gulf	8	3-30	1998	HCMR 2000
Kykclades plateau	16	75-200	1986	NCMR 1989a; Zenetos *et al.,* 1991
Maliakos gulf	16	13-22	1991,1994	NCMR 1994b
Milos island	14	2-72	1986,2009,2010	HCMR 2009d, HCMR 2010b
N. Aegean-Limnos coasts	40	63-1300	1999-2000	KEYCOP 1998-2001
N. Evvoikos gulf	5	26-85	2006	NCMR 1992a; HCMR 2007
Pagassitikos gulf	14	37-99	1997,2008	HCMR 2000b; 2009b
Paros isl	4	8-12	2007	HCMR 2008b
Rodos isl coasts	14	17-24	2003,2009	HCMR 2003d; 2009c; Pancucci-Papadopoulou *et al.,* 1999
S. Evvoikos gulf	34	2-80	1991,1996	NCMR 1992a; NCMR 1997; HCMR 2001b; Simboura *et al.,* 1998 ; Reizopoulou & Zenetos, 2005
Santorini island	14	20-380	1984,2007	NCMR 1989; HCMR 2009a
Saronikos gulf	142	20-400	2000-2010	NCMR 1999; HCMR 2010e; Zenetos *et al.,* 1994 ; Simboura *et al.,* 1995a ; Webb *et al.,* 2009
Sporades isl	13	2-40	1984	UNIV ATHENS, 1985; Simboura *et al.,* 1995b
Strymonikos gulf	10	4-57	1998	HCMR 2000a
Thessaloniki bay	17	7-19	1995	NCMR 1996
Thessaloniki gulf	26	6-28	1991,2001,2002	NCMR, 1994a; NCMR 1996a; HCMR 2001a; 2003e.

Table 2. The predefined categorization of data.

Categories	Ecological Quality Status	Sample size (m^2)	Substratum type	Depth (m)
1	High	<0.1	Homogeneous muds	<35
2	Good	0.1	Heterogeneous (mixed)	35-90
3	Moderate	>0.1	Homogeneous sands/gravel	>90
4	Poor			
5	Bad			

The depth was categorised as follows: category 1 for the infralittoral zone at a depth of 35 m or less, approximately to the lower limit of *Posidonia* beds; category 2 for the circalittoral zone from a depth of 35 m to a depth of 90 m; and category 3 for the lower circalittoral zone from 90 m to 250 m (corresponding to the 'open sea' biocoenoses) and the bathyal zone deeper than 250 m. Category 3 represents the 'open sea' or oceanic zone extending beyond the coastal zone of the WFD and covered exclusively by the MSFD.

To determine the validity or significance of the above categorisation, an analysis of variance was performed for each factor, namely, the depth, sample size and substrata. A multiple range test was applied to identify the categories within each factor that differed significantly from the other categories.

After the statistical determination of the valid categories, similar categories were merged appropriately within each factor to obtain a secondary categorisation of the data corresponding to distinct combinations of conditions or 'ecotypes'. Following this 'ecotype' categorisation, the data were submitted to an analysis of variance of the two indicators, species richness (S) and diversity (H), across the EQS classes. Threshold values of GEnS were set at those levels at which there was a statistically significant difference in the indices values among the EQS classes, especially between class 2 (good) and 3 (moderate) for both indicators. The lower value of the lower quartile of the boxplot of the data (containing 50% of the frequency of values ranging around the mean) was used to set the threshold for class 2. In other cases of non-significant differences, simple statistics (the average and the range) will be presented as an indication of the variance.

Results

The analysis of variance of the sample size, depth and substrata (Table 3) showed that all categories have a significant effect on the indicators. In general, the overall difference among the categories is statistically significant for both S and H except for the effect of sample size on H.

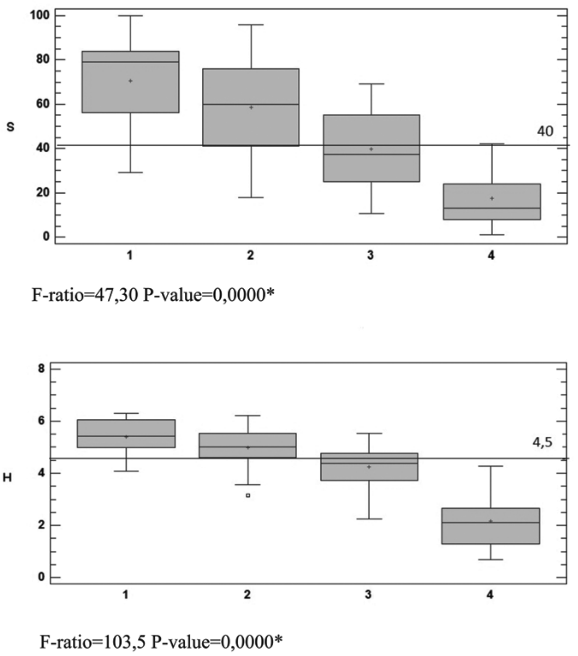
The statistical analysis of the differences in the indices between the pairs of categories shows that depth categories 1 and 2 do not differ significantly and that category 3 differs significantly from the other categories (especially for S). The analysis of substratum type shows that the indicators do not differ significantly for categories 1 and 3 and that category 2 differs significantly from the others.

The analysis of sample size shows that all pairs of categories only differ significantly for the value of the index S.

Based on the above results, the depth zones of 35 m or less and 35-90 m, denoted as 'coastal', will be merged into a single category for further analysis of the data in terms of the differences among the EQS classes. The 'oceanic' or 'open sea' zone will be treated separately. Similarly, the homogeneous substratum types of pure mud and sands (or very fine and very coarse substrata) will be merged into a single category in the analysis of data for EQS differences, whereas the heterogeneous substrata will be analysed separately. The sample-size classes will each be treated separately in the analysis of variance of the indicators among the EQS classes.

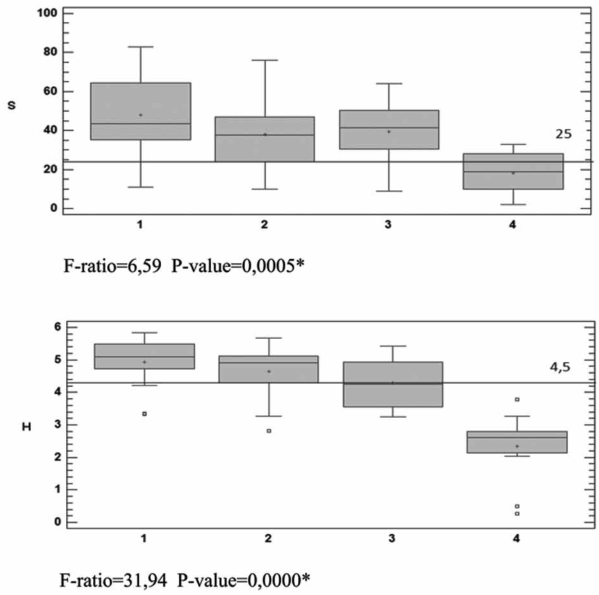
Based on the combined categories for the depth and the type of substratum defined above, the following four 'ecotypes', valid for each of the sample-size categories, emerge from the analysis: Ecotype A, coastal with homogeneous substrata; ecotype B, coastal with heterogeneous substrata; ecotype C, open sea with homogeneous substrata; and ecotype D, open sea with heterogeneous substrata. The boxplots in Figures 1 and 2 show the results of the analysis of variance of the indicators between the EQS classes in the cases for which the differences between the EQS classes are statistically significant for both indicators. The tables accompanying the graphs present the average value and the range of values for each indicator. The differences involving the 'good' and 'moderate' classes will be examined to set GEnS thresholds.

The results of the analysis of variance for S and H across the EQS classes for standard sample size and ecotype B are shown in Figure 1. For this combination of factors, corresponding to the bulk of the data (276 samples), the differences among the classes are highly statistically significant for both indicators. The statistical significance of the differences between pairs of classes was also evaluated for all classes using multiple range tests. These differences proved significant for all pairs of



	S			H		
EQS	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
1	70,53	29,0	100,0	5,40	4,09	6,31
2	58,61	18,0	96,0	4,98	3,14	6,21
3	39,88	10,5	69,0	4,24	2,26	5,52
4	16,01	1,0	42,0	2,24	0,7	4,27

Fig. 1: Boxplots and results of an analysis of variance of S and H across ecological quality classes for standard sample size, coastal zone and heterogeneous substrata (ecotype B).



	S			H		
EQS	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
1	46,0	11,0	83,0	4,87	3,33	5,84
2	39,36	10,0	76,0	4,71	2,81	5,66
3	38,5	9,0	64,0	4,25	3,24	5,43
4	18,17	2,0	33,0	2,34	0,27	3,77

Fig. 2: Boxplots and results of an analysis of variance of S and H across ecological quality classes for standard sample size, open seas zone and heterogeneous substrata (ecotype D).

classes with the exception of the difference between the 'high' and the 'good' class.

Thus, the threshold of the GEnS is set for S at S=40 species and for H at H=4.5 bits based on the lower quartile limit of the box in the 'good' class.

For the standard sample size and ecotype D (open seas and heterogeneous substrata), the analysis (82 observations) showed (figure 2) an overall significant difference for both indicators, but the 'good' class (2) is significantly different from the 'moderate' class (3) for H but not for S. The primary explanation for this result is that the 'good' class in this ecotype includes a group of stations with depths greater than 250 m (bathyal zone), whereas these depths are not represented in the 'moderate' class. More data are needed at this case to arrive at safe threshold setting, but indicatively a tentative threshold value for GEnS can be set at S=25 and H=4.5.

Table 4 shows the results of the analysis of variance for the cases in which the differences among the classes were not significant, or an adequate range of EQS classes did not appear in the data and in which a threshold value could not be set.

The results of the analysis of variance for S and H across the EQS classes for the small sample size and ecotype A (coastal zone and homogeneous substrata) (38 observations) showed no significant difference among the classes for S, whereas a significant difference among the classes was found for H. However, the 'moderate' class is absent (Table 4a).

The result of the analysis of variance for S and H across the EQS classes for the small sample size and ecotype B (52 observations) showed no significant differences for any of the indicators. Only the 'good' and 'moderate' classes appear in the data (Table 4b).

For the standard sample size and ecotype C (open seas and homogeneous very fine substrata) (32 observations), the statistical significance of the differences was not highly significant for either of the indicators (Table 4c). This result is due to the aggregation of stations at depths of approximately 300 m in the 'high' and 'good' classes. The data for these stations represent an excessively low number of species. In contrast, higher species numbers are found in the 'moderate' class, in which only stations at depths of approximately 90 m are pres-

Table 3. Analysis of variance and multiple range tests (MRT) for depth, sediment and sample size and the resulting secondary categorization of data.

	DEPTH		SEDIMENT		SAMPLE SIZE	
	MRT	***F-Ratio P-Value***	**MRT**	***F-Ratio P-Value***	**MRT**	***F-Ratio P-Value***
S	1-2 1-3* 2-3*	16.17* 0.0000	1-2* 1-3 2-3*	67.23* 0.0000	1-2* 1-3* 2-3*	22.07* 0.0000
H	1-2 1-3 2-3*	8.31* 0.0003	1-2* 1-3 2-3*	35.90* 0.0000	1-2 1-3 2-3	0.76 0.4680
Category grouping	1-2 3		1-3 2		No grouping	

** denotes a statistically significant difference.*

ent. This result indicates that a further refinement of the deeper zone of the open sea should distinguish the lower circalittoral zone (90-250 m) from the bathyal zone at depths greater than 250-300 m.

In the case of the large sample size and ecotype B (95 observations), significant differences were not demonstrated (Table 4d). It appears that the differences among the classes are amplified for the larger sample size and increasing sampling effort. For the standard and large sample categories and ecotype A, the differences were not significant. Due to a low number of observations (25 in each case), these results are not presented.

Ecotypes A and C with homogeneous substrata are not represented by sufficient data covering all EQS classes. To obtain statistically substantive results, more data are needed for these cases. The small sample size appears not to be sufficient for threshold setting, whereas larger sample sizes may also mask differences.

The Hellenic area belongs to two MSFD subregions: the Aegean-Levantine subregion, including the Aegean Sea and its embayments, and the Central Mediterranean, including the Ionian Sea subregion with the Ionian and western Peloponnisos coasts. The MSFD guidelines contemplate the possibility of setting regional standards and thresholds for different subregions.

To test for a significant differentiation in the ranges or average values of these indicators between the two different Mediterranean subregions within the Hellenic area, an analysis of variance of S and H according to the location of the sample in the Aegean Sea or the Ionian Sea was applied to the ecotype B data with the standard sample size (to control for other factors influencing the observations). Only the data from the critical 'good' EQS class were analysed (Table 4e). The analysis showed a non-significant difference between the areas. Note that higher values were found for the Ionian Sea. This result may be explained by a superior ecological quality or by the characteristics of the substrata.

The data from the Ionian areas are not abundant (13 stations), and the full range of EQS classes is not observed for the Ionian Sea (only 'high' and 'good' occur). Nevertheless, it appears that the variance of S and H across the EQS classes follows the same pattern in both subregions. Accordingly, the two subregions need not, for the time being, be separated within a given ecotype.

From the above analysis, it appears that statistically significant differences over the entire range of the data, including the Aegean and Ionian Seas, were demonstrated only for ecotypes B and D and the standard sample size. In these cases, an entire range of four EQS classes from 'high' to 'poor' was represented. Thus, safe threshold values could be set.

Based on the above results and the BENTIX index methodology, Table 5 shows the GEnS thresholds in these two ecotypes for S, H and the BENTIX. The reference values for H and S are derived from the maximum values of these indicators observed in our data. Other statistically non-significant results presented may be used indicatively for GEnS assessment. However, an adequate confidence level is not available for these results.

Discussion

Diversity

According to Rice *et al.* (2012), a variety of methods for measuring diversity are available. These methods include beta diversity along a gradient of diversity, alpha diversity at a site, and gamma diversity in multiple habitats. The beta diversity measures include the most frequently used index, the Shannon diversity index. The Shannon diversity index is the most frequently used diversity index in benthic studies conducted in the Hellenic area. The Shannon diversity index, developed from information theory, has been widely used and tested in various environments.

Table 4. Analysis of variance results for species richness (S) and Shanon Diversity (H) in cases with no statistically significant differences a) across ecological quality classes in various ecotypes and sample-size combinations and b) across Aegean and Ionian Sea areas for 'good' ecological status (EQS), ecotype B and standard sample size.

		S			H	
a) Sample size 1, ECOTYPE A						
F=ratio		3.12			23.26	
P-value		0.0387			0.0000*	
EQS	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
1	16.91	11.0	33.0	3.69	3.08	4.69
2	20.0	12.0	36.0	4.19	3.26	5.12
4	14.86	2.0	47.0	2.48	0.24	3.84
b) Sample size 1, ECOTYPE B						
F=ratio		0.03			3.40	
P-value		0.8673			0.0712	
EQS	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
2	36.19	16.0	73.0	4.68	3.5	6.09
3	35.47	13.0	61.0	4.37	3.12	5.23
c) Sample size 2, ECOTYPE C						
F=ratio		0.40			2.75	
P-value		0.67			0.0811	
EQS	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
1	17.56	5.67	30.33	3.64	2.33	4.46
2	22.03	8.67	61.0	3.87	3.04	5.18
3	20.47	12.0	29.0	3.31	2.38	3.79
d) Sample size 3, ECOTYPE B						
F=ratio		1.84			4.56	
P-value		0.164			0.0128	
EQS	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
1	71.46	27.0	143.0	5.075	3.9	6.2
2	60.24	15.0	126.0	4.76	2.18	5.99
3	58.08	12.0	91.0	4.42	2.23	5.38
e) Sample size 2, ECOTYPE B, GOOD EQS						
F=ratio		1.47			2.82	
P-value		0.2281			0.0908	
AREA	*Average*	*Minimum*	*Maximum*	*Average*	*Minimum*	*Maximum*
Aegean	55.05	18.0	96.0	4.90	3.14	6.21
Ionian	65.67	49.0	95.0	5.31	4.81	5.75

The WFD guidance document (EC, 2003) for typology, reference conditions and classification states that 'methods combining composition, abundance, and sensitivity may be the most promising'. Moreover, according to Rice *et al.* (2012), communities with GEnS are those with a few abundant species and many rare ones. Such communities show a high resilience potential in the face of moderate pressures because biodiversity buffers ecosystem processes and, through these processes, the ecosystem services that can be used sustainably (Loreau *et al.*, 2002).

However, the values of community diversity are influenced by the sample size, sampling methodology and species identification procedures. Moreover, seasonal

Table 5. Threshold values and reference conditions for Good Environmental Status (GEnS) for Species richness (S) and Shannon Diversity (H) in Ecotypes B and D.

Ecotype B: coastal, heterogeneous substrata			
	S	H	BENTIX
GEnS thresholds	40	4.5	3.5
Reference conditions	100	>5	6
Ecotype D: Open seas, heterogeneous substrata			
GEnS thresholds	25	4.5	3.5
Reference conditions	>80	>5	6

natural variability and habitat type influence diversity and species richness. For these reasons, it is generally recommended that diversity and species richness be used with caution in ecological classification (Reiss & Kröncke, 2005; Salas *et al.*, 2006).

According to the present study, sample size did not affect diversity significantly (Table 3). In contrast, sample size had a significant effect on species richness. It is probable that this difference occurred because the evenness component of diversity is independent of sample size. In contrast, it was shown that the significant influences on the variance of diversity include substratum type as the primary influence and depth as the secondary influence. The significant differences occurred between specific combinations of these factors or 'ecotypes'.

For each ecotype, the variance in diversity among the EQS classes was statistically important to a higher degree and at a higher frequency than the variance in species richness (Figs 1, 2 and Table 4). This result indicates that H is a more reliable indicator of the EQS than species richness.

Based on a large data series, Zenetos & Simboura (2001) and (with a modification to the boundary between 'good' and 'high') Simboura & Zenetos (2002) divided the Shannon-Wiener diversity index values into five categories. These authors' analyses applied primarily to mixed-sediment marine benthic habitats in Hellenic coastal waters. The class boundary between the 'moderate' and 'good' classes, corresponding to the GEnS threshold, was then set at 4 bits per unit.

In a recent work, Subida *et al.* (2010; 2012), elaborating the results from a large dataset of soft-bottom macrofauna samples collected in several Mediterranean coastal areas affected by different ranges of organic enrichment, observed that diversity measures did not show monotonic patterns of response to the gradient of organic content, particularly at the low end of its range, whereas strong correlations were found between indicator taxa indices and a anthropogenic pressure-indicator gradient. This non-linear response of diversity to a pressure gradient may weaken the relationship among the multimetrics containing diversity, and the anthropogenic pressure indicators.

The absence of a monotonic response of diversity to pressure gradients may be explained by the Pearson & Rosenberg (1978) model of the variation of species-abundance-biomass across a pollution gradient. Specifically, a transition zone between the disturbed and normal state occurs after the ecotone point. In this zone, the community often reaches a maximum in the number of species present in both adjacent environments (enriched and less enriched), whereas the abundance of species declines to the steady-state level usually found in normal communities. In these cases, diversity may be significantly high in a community that is still disturbed. These cases of disturbed communities with high diversity values may be detected by carefully examining the abundances of individual indicator species and the proportion of sensitive species to tolerant species.

In Mediterranean coastal water ecosystems that are naturally poor in sediment organic matter content, indicator taxa indices such as the MEDOCC (from Mediterranéo Occidental) (Pinedo & Jordana, 2008), the Benthic Opportunistic Polychaetes Amphipods index (BOPA) (Dauvin & Ruellet, 2007), the AZTI's Marine Biotic Index (AMBI) (Borja *et al.*, 2000) or BENTIX (Simboura & Zenetos, 2002) appear to furnish a more reliable account of the response of benthic communities to moderate increments of organic content than diversity indices (Subida *et al.*, 2012).

The lack of statistically significant differences in diversity among the classes of EQS in several cases of ecotypes may be attributed primarily to the lack of sufficient data for all classes within each ecotype, but this result may also be due to the absence of a monotonic response of diversity to environmental pressures.

Species richness

The number of species in a benthic community varies greatly with depth and sediment type. A typical trend observed in the Mediterranean is a significant decrease in species number with depth. Food availability, related to depth, may also have a substantial influence on the levels of biodiversity.

In this study, substratum type was the second most significant factor after disturbance in terms of the relative influence on the species richness in a given biotope. Different communities (benthic assemblages associated with certain sediment types/depths) exhibit different species numbers. It is well established in benthic ecology that the sediment composition, particularly the relative contribution of finer or coarser particles to the homogeneity/heterogeneity of the substratum, the diversity of microhabitats and the retention of food resources, plays an important role in benthic community composition and structure (Gray, 1974; Gambi & Giangrande, 1986).

The present study showed that the depth and type of substratum had a significant effect on species richness but affected diversity relatively less (Table 3).

This study also showed that species richness differed between two general categories of substrata: homogeneous substrata and heterogeneous or mixed sediments. The first substratum category, consisting of homogeneous sediments, includes plain muddy sediments with mud (silt and clay) content greater than 90% and without significant amounts of biogenic fragments, but this category also includes pure sands with little or no fine particles or biogenic detritus. Muddy sediments are usually found in enclosed, shallow basins receiving riverine inputs or in bathyal plains, where terrigenous mud is accumulated. The second substratum category refers primarily to mixed sediments with a combination of fine and coarser materials.

In addition, a primary pattern in biodiversity, noted universally, is that the number of species asymptotically increases with the area sampled. The number of taxa and the diversity discovered in an area are proportional to the sampling and taxonomic effort exerted. As the results of this study show, it appears that small samples are not adequate for detecting the maximum potential species richness and diversity, especially at a high EQS level (Table 4a). The number of species (S) found in a standard sampling unit can be a reliable measure of environmental stress.

The differences in species richness across EQS classes were always, as the F-ratios and P-values in Table 4 indicate, less significant than the diversity differences. This effect reflects the tendency for diversity, which encompasses evenness and is less dependent on sample size, substratum type and depth (Table 3), to furnish a better description of the EQS gradient than that obtained from S. Thus, Shannon diversity may be a more reliable indicator than species richness. As shown by the boxplots, the range of S in each EQS class is often wider than the corresponding range of diversity, indicating the large variance of S within each ecotype and EQS.

GEnS thresholds and standards using BENTIX, H and S

The above analysis furnishes clear indications that the primary factor determining the range of indicator values, in addition to anthropogenic pressure and sample size, is the substratum structure and composition. Depth is a secondary factor, as has been well documented in the literature. For this reason, the entire data set from the Hellenic seas was categorised and statistically analysed to assess possible indicative thresholds for the 'good' class. The analysis also showed that small geographical scales within the same basin, at least for the Hellenic Seas, do not play any significant role in determining the range of indicator values. An analysis of variance and its graphical representation with boxplots showed that the threshold values for the 'good' class were statistically significant only in the case of ecotype B for both S and H and in the case of ecotype D for H. It is noteworthy that the threshold for H and ecotype D is identical to the threshold of H for ecotype B, although the threshold for S and ecotype B is almost double compared to the indicative S threshold for ecotype D. This can be explained by the observations of Karakassis & Eleftheriou (1977) from the continental shelf of Kriti and of Simboura *et al.* (2000) from the Kyklades plateau, that in the oligotrophic eastern Mediterranean the evenness of distribution increases with depth, while species richness is reduced. Thus, in relatively undisturbed deep zone communities, species richness maybe low while diversity may not be significantly influenced.

Generally, H proved more relevant to EQS than S. The threshold value of diversity set according to the biostatistical method is more stringent than the scheme of Zenetos & Simboura (2001), which is based on expert judgment.

The relatively low agreement of these two indicators with EQS may be explained by the dependence of these indicators on different habitats or ecotypes, as also noted by Borja *et al.* (2010), and by the lack of strong monotonic relationships with anthropogenic pressure gradients.

Even in the case of a statistically significant threshold (ecotype B) in the 'good' class, the overlap of the boxplots among classes shows that the evaluation of EQS should be conducted with caution and also in combination with the results for other indicators.

However, in the case of statistically non-significant differences in certain ecotypes, the range of the main body (50%) of values around the mean may also be useful for the evaluation of ecotype status.

A threshold value (Table 5) is set based on the rationale that if the value of an index reaches or surpasses the threshold, a GEnS can then be assumed if the other indices are also considered. However, if an index value is lower than the threshold, a GEnS cannot be excluded because lower values of the index may be justified by many other factors, including sampling effort, substratum characteristics (especially concerning the percentages of fine particles relative to coarser particles), the presence of other materials, seasonal variations or the level of taxonomic expertise. It can reasonably be assumed that the normality of the distribution of these indices is driven by the variation in the above factors, especially by the particle composition of the sediments.

Note that the threshold values given in Table 5 for diversity and species richness correspond to a sample size of 0.1 m^2, heterogeneous sediments and a high level of taxonomic expertise.

As Borja *et al.* (2010) showed, the global status of an area assessed by different ecological quality elements may be assessed with a weighting system that sums or integrates the results for the different indicators on the global scale of the Ecological Quality Ratio (EQR) developed under the WFD. Borja *et al.* (2011) show how the Sea-floor Integrity descriptor can be assessed by averaging the values of its criteria or indicators according to EC(2010),then weighting their contributions to the global area status and summing the EQR results of all GEnS descriptors. In the same context, Rice *et al.* (2012) note that the assessment of the GEnS in an area should be an integrative process incorporating all Sea-floor indicators and should not follow a weighted scoring system that averages the results obtained from each indicator.

In the case of non-discrete EQR values for H and S, a generally good rule is that at least two of the three indices chosen should pass the threshold values to assume a GEnS.

However, cases of moderate EQS, as assessed by the BENTIX index with values of diversity and species richness higher that the thresholds set (indicating GEnS), should be examined carefully for underlying ecotonal zone (not 'good' environmental status) and/or substratum/habitat particularities (e.g., the proximity of *Posidonia* meadows).

A factorial analysis incorporating all three indicators (H, S and BENTIX) would distort and obscure the actual state of the benthic community.

The M-AMBI index, which incorporates diversity with a biotic index, has been shown to overestimate the status of Hellenic ecotonal zones whose status is near the border between 'good' and 'moderate' (Simboura & Argyrou, 2010) and to produce mismatches in the assessment of ecological status with other biotic indices in Mediterranean coastal ecosystems (Subida *et al.*, 2012). This result is attributed, in part, to the double weighting of diversity (directly as H and indirectly as S) by the index. Compared with the other indices tested in the Mediterranean ecoregion, however, the M-AMBI index showed the closest agreement with BENTIX in the critical area of 'moderate' to 'good' (GIG, 2012).

An analysis of inter-relationships among the biotic indices and those including diversity, such as M-AMBI, over the global Mediterranean dataset (Subida *et al.*, 2012) showed that the best correlations were observed for the MEDOCC index with AMBI or BENTIX, depending on the region, whereas BOPA showed high correlations with AMBI. Significant correlations were also found between BOPA, MEDOCC and BENTIX. M-AMBI was more highly correlated with its diversity components than with AMBI, and, consequently, M-AMBI was less highly correlated with the pressure gradients than with AMBI. If the indices were tested only on data from the Eastern Mediterranean, the best agreement was between BENTIX and MEDOCC, followed by M-AMBI, whereas the highest percentage of mismatches was found between AMBI and BOPA (Simboura & Argyrou, 2010).

However, Grémare *et al.* (2009) highlighted a weakness in the way biotic indices such as the AZTI Marine Biotic Index (AMBI) and the Benthic Quality Index (BQ-I_{ES}) (Rosenberg *et al.*, 2004) assess sensitivity/tolerance levels over large geographical areas and habitats.

Borja *et al.* (2010) recommend the use of the principle of reference conditions defined according to WFD as an adaptation for setting the environmental quality standards for MSFD indicators as well.

An approach to establishing the reference levels for H and S was developed to use such metrics in relation to multimetric indices (e.g., M-AMBI) for the Hellenic ecosystems. These values were set as H=6 and S=110-120 for mixed and phytal benthic sediments and H=5 and S=40 for muddy sediments (greater than 80-85% mud) (Simboura & Reizopoulou, 2008). These values correspond to the median values of the best available sites increased by approximately 10% of the absolute difference between the lower anchor and the median value and were set exclusively for use with the M-AMBI software.

The reference conditions under WFD correspond to the values under very minor anthropogenic disturbance (pristine sites). The thresholds for GEnS and the standards for 'high' environmental status for Diversity (Table 5) generally agree with the ranges given in Zenetos & Simboura (2001) and (UNEP/MAP, 2004) for the Mediterranean, but the GEnS threshold set herein (4.5) is somewhat more ambitious.

The above analysis on the "behaviour" of each diversity indicator will help to assess the environmental condition of benthic communities in conjunction with the consideration of many other factors included in the Commission Decision (EC, 2010) and the Management Group report (Cardoso *et al.*, 2010), including species dominance, biological traits analysis, trophodynamics, contaminants, substratum condition, and the size structure of benthic communities. These factors are also essential for the assessment of GEnS based on specific criteria.

The issue of regional scales in the implementation of the MSFD is clarified by previous experience with the WFD. The results of this previous experience show that for both the benthic macroinvertebrate element and the macroalgae element, no differentiation in typology was found to affect the application and boundaries of the various benthic indices tested. Only the phytoplankton biomass quality element was relevant to certain typology within the Mediterranean, related primarily to salinity and freshwater inputs (GIG, 2008). According to Borja *et al.* (2010), experience with typology under the WFD should also be beneficial for the implementation of the MSFD in relation to regional settings and standards. The model of the succession of macroinvertebrate ecological groups is the foundation of the design, structure and optimum performance of the various biotic indices. As Simboura & Argyrou (2010) indicate, this model can only be related to a certain extent to this factor of freshwater input affecting salinity levels within the Mediterranean. The factor of salinity affected by freshwater inputs may also affect the threshold setting of the above benthic indicators of species richness and diversity within the MSFD. However, this factor shows almost no variation in the marine waters studied within the Hellenic area. Other natural factors, such as substratum composition and depth related to food availability, appear to configure the levels and thresholds for these indicators in the area studied.

Acknowledgements

The research leading to these results was supported by funding from the European Community's Seventh Framework Programme ([FP7/2007-2013]) under grant agreement n° 287600 - PERSEUS project (Policy-oriented marine Environmental Research for the Southern EUropean Seas). The authors gratefully acknowledge the contribution of the two referees who critically revised the manuscript and provided their constructive comments and suggestions.

References

Bellan-Santini, D., 1994. Les biocénoses benthiques. I. Définition des principaux concepts utilises. p. 48-49. In: *Les biocénoses marines et littorales de Méditerranée. Synthèse, menaces et perspectives.* D. Bellan-Santini, J.C. Lacaze & C. Poizat (Eds). Muséum National d'Histoire Naturelle (Paris).

Bogdanos, C., Simboura, N. & Zenetos, A., 2002. The benthic fauna of Geras Gulf, (Lesvos isl. Greece): inventory, distribution and some zoogeographical considerations. *Hellenic Zoological Archives*, 6: 1-22.

Borja, Á., Franco, J. & Pérez, 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.

Borja, Á., Franco, F., Valencia, V., Bald, J., Muxica, I. *et al.,* 2004. Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. *Marine Pollution Bulletin,* 48 (3-4): 209-218.

Borja, Á., Elliott, M., Carstensen, J., Heiskanen, A.-S. & van de Bund, W., 2010. Marine management – towards an integrated implementation of the European Marine Strategy Framework and the Water Framework Directives. *Marine Pollution Bulletin*, 60 (12): 2175-2186.

Borja, Á., Galparsoro, I., Irigoien, X., Iriondo, A., Menchaca, I. *et al.,* 2011. Implementation of the European Marine Strategy Framework Directive: A methodological approach for the assessment of environmental status, from the Basque Country (Bay of Biscay). *Marine Pollution Bulletin*, 62 (5): 889-904.

Cardoso, A.C., Cochrane, S., Doerner, H., Ferreira, J.G., Galgani, F. *et al.,* 2010. Scientific support to the European Commission on the Marine Strategy Framework Directive: Management group report. *JRC Scientific and Technical Reports*. Luxembourg, Office for Official Publications of the European Communities, 57 pp.

Dauvin, J.C. & Ruellet, T., 2007. Polychaete/amphipod ratio revisited. *Marine Pollution Bulletin*, 55 (1-6): 215-224.

EC, 2003. *Guidance on typology, reference conditions and classification systems for transitional and coastal waters*. Produced by: CIS Working Group 2.4. (Coast). Common Implementation Strategy of the Water Framework Directive, European Commission, 116 pp.

EC, 2010. Commission Decision of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters (notified under document C(2010) 5956)(2010/477/EU). *Official Journal of the European Union*, L232: 14-24.

Flo, E., Camp, J. & Garcés, E., 2011. *Assessment pressure methodology: Land Uses Simplified Index (LUSI). BQE Phytoplankton*. Spain, Catalonia. Work document.

Gambi, M.C. & Giangrande, A., 1986. Distribution of soft-bottom polychaetes in two coastal areas of the Tyrrhenean Sea (Italy): structural analysis. *Estuarine Coastal & Shelf Science*, 23: 847-862.

GIG, 2008. WFD intercalibration technical report. Part 3 – Coastal and Transitional Waters. Sect. 2 – Benthic invertebrates. Four parts: Mediterranean GIG; Black Sea GIG; North East Atlantic GIG; and Baltic GIG. http://circa.europa.eu/Public/irc/jrc/jrc_eewai/library.

GIG, 2012. Final Report – macroinvertebrate subgroup Coastal GIGs. MED-GIG (Mediterranean coastal).

Gray, J.S., 1974. Animal-sediment relationships. *Oceanography &* Marine *Biology:* Annual Review, 12: 223-261.

Grémare, A., Labrune, C., Vanden Berghe, E. Amouroux, J.M., Bachelet, G. *et al.,* 2009. Comparison of the performances of two biotic indices based on the MacroBen database. *Marine Ecology Progress Series*, 382: 297-311.

HCMR 2008. Development of the monitoring network for the ecological status quality of the inland, transitional and coastal waters of Greece-Classification of their ecological quality status. Typo-characteristic reference conditions. Development of a monitoring network according to the WFD 2000/60/EC. HCMR-MINENV. Coordination of Coastal & Transitional waters part. Scientific responsible for Coastal waters part. Technical Report, (In Greek).

Karakassis, I., 1991. *Contribution to the study of the benthic ecosystem of the continental shelf of Crete*. PhD Thesis. University of Crete, 195 pp. (In Greek).

Karakassis, I., & Eleftheriou, A., 1997. The continental shelf of Crete: structure of macrobenthic communities. *Marine Ecology Progress Series*, 160: 185-196.

KEYCOP, 1998-2001. KEYCOP: EU MAST-III EC Project MAS3-CT97-0148 (1998-2001). Key coastal processes in the mesotrophic Skagerrak and the oligotrophic Northern Aegean: a comparative study (KEYCOP). WP Benthic ecosystem structure of the N. Aegean.

Labrune, C., Amouroux, J.M., Sarda, R., Dutrieux, E., Thorin, S. *et al.,* 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. *Marine Pollution Bulletin*, 52 (1): 34-47.

Loreau, M., Naeem, S. & Inchausti, P., 2002. *Biodiversity and ecosystem functioning. synthesis and perspectives*. Oxford, UK, Oxford University Press, 283 pp.

Muxika, I., Borja, Á. & Bald, J., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European water framework directive. *Marine*

Pollution Bulletin, 55: 16-29.
Pancucci-Papadopoulou, M.A. Simboura, N., Zenetos, A., Thessalou-Legaki, M. & Nicolaidou, A., 1999. Benthic invertebrate communities of NW Rodos island (SE Aegean Sea) as related to hydrological and geographical location. *Israel Journal of Zoology*, 45: 371-393.
Pearson, T.H. & Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography &* Marine *Biology:* Annual Review*,* 16: 229-311.
Pérès, J.M. & Picard, J., 1964. Nouveau manuel de bionomie benthique de la Mer Méditerrané. Recueil des Travaux de la Station Marine d'Endoume, 47 (31): 3-137.
Pinedo, S. & Jordana, E., 2008. Spain (Catalonia and Balearic Islands). p. 62-70. In: *Water Framework Directive Intercalibration Technical Report. Part 3: Coastal and Transitional Waters*. Carletti, A. & Heiskanen, A.-S. (Eds). JRC Scientific and Technical Reports.
Reiss, H. & Kröncke, I., 2005. Seasonal variability of benthic indices: an approach to test the applicability of different indices for ecosystem quality assessment. *Marine Pollution Bulletin*, 65: 253-274.
Reizopoulou, S. & Zenetos, A., 2005. A preliminary study of an eastern Mediterranean coastal ecosystem: Summer Resorts and Benthic ecosystems. *Mediterranean Marine Science*, 6 (1): 41-50.
Rice, J., Arvanitidis, C., Borja, Á., Frid, C., Hiddink, J. *et al.,* 2010. Marine Strategy Framework Directive – Task Group 6 Report Seafloor integrity. Luxembourg, Office for Official Publications of the European Communities, 73 pp.
Rice, J., Arvanitidis, C., Borja, A., Frid, C., Hiddink, J. *et al.,* 2012. Indicators for sea-floor integrity under the European Marine Strategy Framework Directive. *Ecological Indicators*, 12: 174-184.
Rosenberg, R., Blomqvist, M., Nilsson, H.C., Cederwall, H. & Dimming, A., 2004. Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Marine Pollution Bulletin*, 49: 728-739.
Salas, F., Patrício, J., Marcos, C., Pardal, M.A., Pérez-Ruzafa, A. & Marques, J.C., 2006. Are taxonomic distinctness measures compliant to other ecological indicators in assessing ecological status? *Marine Pollution Bulletin*, 52: 162-174.
Shannon, C.E. & Weaver, W., 1963. *The mathematical theory of communication*. Urbana, USA, University of Illinois Press, 117 pp.
Simboura, N., Zenetos, A., Panayotidis, P. & Makra, A., 1995a. Changes of benthic community structure along an environmental pollution gradient. *Marine Pollution Bulletin*, 30 (7): 470-474.
Simboura, N., Zenetos, A., Thessalou-Legaki, M., Pancucci, M.A., & Nicolaidou, A., 1995b. Benthic communities of the infralittoral in the N. Sporades (Aegean sea): a variety of biotopes encountered and analysed. *PSZNI Marine Ecology*, 16 (4): 283-306.
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. *PSZNI Marine Ecology,* 19 (4): 293-309.
Simboura**,** N., Nicolaidou, A., & Thessalou-Legaki, M., 2000. Polychaete communities of Greece: an ecological overview. *PSZNI Marine Ecology*, 21 (2): 129-144.
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.
Simboura, N., Panayotidis, P. & Papathanassiou, E., 2005. A synthesis of the Biological Quality Elements for the implementation of the European Water Framework Directive in the Mediterranean Ecoregion: the case of Saronikos Gulf. *Ecological Indicators*, 5: 253-266.
Simboura, N., Argyrou, M., 2006. Implementation of the WFD Directive in Cyprus: application of the Bentix index in Limassol Bay (Cyprus). Abstracts of the 8th Hellenic Symposium of Oceanography and Fisheries, Thessaloniki, June 2006, p.77.
Simboura, N. & Reizopoulou, S., 2007. A comparative approach of assessing ecological status in two coastal areas of Eastern Mediterranean. *Ecological Indicators,* 7: 455-468.
Simboura, N. & Reizopoulou, S., 2008. An intercalibration of classification metrics of benthic macroinvertebrates in coastal and transitional ecosystems of the Eastern Mediterranean ecoregion (Greece). *Marine Pollution Bulletin*, 56: 116-126.
Simboura, N. & Argyrou, M., 2010. An insight into the function of benthic classification indices tested in Eastern Mediterranean coastal waters. *Marine Pollution Bulletin*, 60 (5): 701-709.
Subida, M.D., Drake, P., Jordana, E., Mavriè, B., Pinedo, S. *et al.,* 2010. Implications of non-linear responses of diversity to disturbance gradients in the assessment of the European Water Framework Directive ecological status integrative tools and methods in assessing ecological quality in estuarine and coastal systems worldwide. In: *ECSA 47 Symposium (Estuarine & Coastal Sciences Association) 14-19 September, 2010, Figueira da Foz, Portugal*.
Subida, M.D., Drake, P., Jordana, E., Mavriè, B., Pinedo, S. *et al.,* 2012. Response of different biotic indices to gradients of organic enrichment in Mediterranean coastal waters: implications of non-monotonic responses of diversity measures. *Ecological Indicators*, 19: 106-107.
UNEP/MAP/MED POL, 2004. Guidelines for the development of ecological status and stress reduction indicators for the Mediterranean region. *MAP Technical Reports Series, No. 154*. Athens.
UNIVERSITY OF ATHENS, 1985. *Quantitative study of the benthic fauna of the Northern Sporades*. Final Report. University of Athens.
Van Hoey, G., Borja, A., Birchenough, S., Buhl-Mortensen, L., Degraer, S. *et al.,* 2010. The use of benthic indicators in Europe: from the Water Framework Directive to the Marine Strategy Framework Directive. *Marine Pollution Bulletin*, 60 (12): 2187-2196.
Zenetos, A. & Papathanassiou, E., 1989. Community parameters and multivariate-analysis - as a means of assessing the effects of tannery effluents on macrobenthos. *Marine Pollution Bulletin*, 20 (4): 176-181.
Zenetos, A. & Simboura, N., 2001. Soft bottom benthic indicators. *Rapports et Procès-Verbeaux des Réunions Commission international pour l'Exploration scientifique de la Mér Méditerranée*, 36: 339.
Zenetos, A., Papathanassiou, E. & Van Aartsen, J.J., 1991.

Analysis of benthic communities in the Cyclades plateau (Aegean Sea) using ecological and paleoecological data sets. *PSZNI Marine Ecology,* 12 (2) : 123-137.

Zenetos, A., Simboura, N., & Panayotidis, P., 1994. Effects of sewage on the distribution of benthic fauna in Saronikos Gulf. *UNEP-MAP Technical Reports Series,* 80: 39-72.

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.

Webb, T., Aleffi, I.F., Amouroux, J.-M., Bachelet, G., Degraer, S. *et al.,* 2009. Macroecology of the European soft sediment benthos: insights from the Macroben database. *Marine Ecology Progress Series*, 382: 287-296.