

## Mediterranean Marine Science

Vol 16, No 1 (2015)

Vol 16, No 1 (2015)



### Assessment of the environmental status in Hellenic coastal waters (Eastern Mediterranean): from the Water Framework Directive to the Marine Strategy Water Framework Directive.

N. SIMBOURA, M. TSAPAKIS, A. PAVLIDOU, G. ASSIMAKOPOULOU, K. PAGOU, H. KONTOYANNIS, C. ZERI, E. KRASAKOPOULOU, E. ROUSSELAKI, N. KATSIARAS, S. DILIBERTO, M. NALETAKI, K. TSIAMIS, V. GERAKARIS, P. DRAKOPOULOU, P. PANAYOTIDIS

doi: [10.12681/mms.960](https://doi.org/10.12681/mms.960)

#### To cite this article:

SIMBOURA, N., TSAPAKIS, M., PAVLIDOU, A., ASSIMAKOPOULOU, G., PAGOU, K., KONTOYANNIS, H., ZERI, C., KRASAKOPOULOU, E., ROUSSELAKI, E., KATSIARAS, N., DILIBERTO, S., NALETAKI, M., TSIAMIS, K., GERAKARIS, V., DRAKOPOULOU, P., & PANAYOTIDIS, P. (2014). Assessment of the environmental status in Hellenic coastal waters (Eastern Mediterranean): from the Water Framework Directive to the Marine Strategy Water Framework Directive. *Mediterranean Marine Science*, 16(1), 46–64. <https://doi.org/10.12681/mms.960>

## Assessment of the environmental status in the Hellenic coastal waters (Eastern Mediterranean): from the Water Framework Directive to the Marine Strategy Framework Directive

N. SIMBOURA<sup>1</sup>, M. TSAPAKIS<sup>2</sup>, A. PAVLIDOU<sup>1</sup>, G. ASSIMAKOPOULOU<sup>1</sup>, K. PAGOU<sup>1</sup>,  
H. KONTOYIANNIS<sup>1</sup>, CH. ZERI<sup>1</sup>, E. KRASAKOPOULOU<sup>3</sup>, E. ROUSSELAKI<sup>1</sup>,  
N. KATSIARAS<sup>1</sup>, S. DILIBERTO<sup>2</sup>, M. NALETAKI<sup>2</sup>, K. TSIAMIS<sup>1</sup>, V. GERAKARIS<sup>1</sup>,  
P. DRAKOPOULOU<sup>1</sup> and P. PANAYOTIDIS<sup>1</sup>

<sup>1</sup> Institute of Oceanography, Hellenic Centre for Marine Research, 46.7 Km Athens-Sounio Ave, Mavro Lithari, P.O. Box 712, 19013, Anavissos, Attika, Greece

<sup>2</sup> Institute of Oceanography, Hellenic Centre for Marine Research, P.O. Box 2214, 71003, Heraklion, Greece

<sup>3</sup> University of the Aegean, Department of Marine Sciences, 81100, Lesvos, Greece

Corresponding author: [msim@hcmr.gr](mailto:msim@hcmr.gr)

Handling Editor: Argyro Zenetos

Received: 20 June 2014; Accepted: 29 June 2014; Published on line: 28 November 2014

### Abstract

A tested methodology is presented to assess the environmental status *Sensu* on the Marine Strategy Framework Directive (MSFD) based on the data obtained from the monitoring of water quality in the Hellenic coastal waters within the Water Framework Directive (WFD). A decision tree developed by Borja *et al.* (2004) for integrating WFD results was applied after some adaptations. Modifications were included to evaluate the physicochemical status based on the eutrophication index developed for the Eastern Mediterranean waters. Results regarding the hydromorphological, physicochemical and biological elements are presented. The chemical status was assessed based on the concentrations of the heavy metals in water. Evaluation of the biological quality was based on the use of metrics developed for the phytoplankton biomass, benthic macroinvertebrates and macroalgae updated to accommodate the MSFD needs. Results from the integrative status of the water bodies were validated by correlating the classification results with a pressure index and environmental indicators in the water column and sediment. Following this decision tree the majority of stations expected to be at risk for achieving the 'good' status were found in the 'moderate' status. The benthos was found to be the element showing the closest agreement with the integrated final status having an increased weightage in the decision tree. The benthos quality and, in some limited cases, the eutrophication index largely determined the final status. The highest disagreement with the integrative classification was revealed by the macroalgae. All the indicators used correlated with the water and sediment parameters, although the benthos correlated better with the sediment factors while the phytoplankton and eutrophication index correlated with the water column parameters.

**Keywords:** Water Framework Directive, Marine Strategy Framework Directive, Eastern Mediterranean, Integrative environmental status, biological quality, physicochemical quality.

### Introduction

The European Water Framework Directive (WFD) (2000/60/EC) (EC, 2000) uses the Biological Quality Elements (BQE) as the basic quality elements for the evaluation of the ecological status of the water bodies, whereas the other elements (hydromorphological and physicochemical) are only used to support the BQEs. The Marine Strategy Framework Directive in turn is a more holistic Directive and uses eleven Descriptors with several indicators covering the ecological, physical, chemical and anthropogenic components of the ecosystem that need to be integrated at the indicator and descriptor levels (Van Hoey *et al.*, 2010).

However, all the biological and supportive hydro-morphological and physicochemical elements of the WFD address many of the MSFD indicators and descrip-

tors and the two Directives largely overlap (Zampoukas *et al.*, 2012). An informative work by Borja *et al.* (2010) presents a system of applying the experience gained from the WFD to implement the MSFD. This work outlines the points of overlap and conflict between the two directives.

Borja *et al.*, (2004) described a methodology (further updated in Borja *et al.* 2009a) that integrates the biological elements together with the hydro-morphological and physicochemical elements (including pollutants) into a unique quality assessment within the Basque Country using only the WFD elements. In this way the approach of 'One-Out, All-Out' (OOAO) recommended by the WFD Guidance (EC, 2003) for integrating across the elements of biological quality is circumvented and a more integrated synthesis is possible. This "worst status element" approach has been seriously criticized for increasing the

probability of committing a false positive error (erroneously downgrading a waterbody to a worse class) (Borja & Rodríguez, 2010). Similarly, Borja *et al.* (2009b) on reviewing the challenge of assessing the ecological integrity in marine waters, suggest that simple approaches such as the 'One-Out, All-Out' principle of the WFD, which takes the final quality of a water body from the worst rated element, may be a useful starting point, but one that should eventually be avoided. Several methodological approaches recommended for integrating the assessment of the MSFD indicators and descriptors have been reviewed (Prins *et al.*, 2013) including the decision trees.

With the acquisition of more data on the other descriptors, integration schemes for all the descriptors and indicators will become possible, for example the weighted rating system proposed by Borja *et al.* (2011) for the assessment of the environmental status in the case of the Basque country giving specific weights to those associated with the significant pressures. The environmental integrity of an aquatic system should be evaluated using all the information available, including as many elements of the biological ecosystem as is reasonable, by using an ecosystem-based assessment approach.

However, the transition from the WFD to the MSFD should be seamless, without any conflicts in the assessment (Borja *et al.*, 2010), and therefore, a starting base is necessary. To enable this, the decision tree integrating the WFD results into the first MSFD assessment is presented here.

On reviewing the objectives and requirements of marine water quality monitoring, Karydis & Kitsiou (2013) highlighted the paucity of marine monitoring programs in which the information provided is assimilated into the integrated assessments and ultimately attributed to the requirements and complexity of such an approach.

In the present work, the first year results of the Hellenic coastal waters monitoring network for the WFD were used following the methodological approach proposed by Borja *et al.* (2004) for integrating the results of the various biological and physicochemical elements into an integrative environmental status.

### **The data set**

The data set used originated from one seasonal sampling of the 44 stations of the national monitoring network designed for the implementation of the WFD in the coastal waters. This first sampling cruise of the monitoring network, during which the data presented were collected, was conducted in April and May 2012, in 44 stations by the R/V PHILIA of HCMR ownership.

Although the results presented here refer to only one season, they are considered to be representative of the areas sampled as, according to the integrative methodology applied, the most heavily weighted element is the benthic macroinvertebrates, which is sampled only once per year and during the first cruise presented here.

## **Methodologies used and elements measured**

### *Hydromorphological and physicochemical conditions*

Current speed and direction were measured by employing an Acoustic Doppler Current Profiler (ADCP), and the CTD (Conductivity, Temperature, Depth) profiles were recorded. Transparency was measured using a Secchi disk depth of disappearance.

The sediment was sampled with a Smith McIntyre benthic grab with a 0.1m<sup>2</sup> sampling surface used also for sampling of the benthic macroinvertebrates. Grain size analysis was performed according to Folk (1954). The total nitrogen and organic carbon were measured in an elemental (CHN) analyzer following the methodology outlined by Verardo *et al.* (1990).

Oxygen and nutrient samples were collected from discrete standard depths of 2m, 10m, 20m, 50m, and the bottom layer depending on the station depth. The Winkler method was used to determine the oxygen using the standard methods for nutrient measurements: (Murphy & Riley, 1955, for silicate; Strickland & Parsons, 1977, for nitrate-nitrite; Murphy & Riley, 1962, for phosphate; and Koroleff, 1970, for ammonium).

The physicochemical quality regarding the condition of the combined nutrients was estimated using the Eutrophication index of Primpas *et al.*, 2010, explained in further detail below.

The seawater samples for Total Nitrogen (TN) and Total Phosphorus (TP) were collected and analyzed in the lab by persulfate wet-oxidation according to Pujo-Pay & Raimbault (1994) and Raimbault *et al.* (1999a).

Particulate Organic Carbon (POC) was measured in a Thermo Scientific FLASH 2000 CHNS elemental analyzer according to the methodology suggested by Cutter & Radford-Knoery (1991) and Verardo *et al.* (1990), and Particulate Organic Phosphorus (POP) was determined using the persulfate wet-oxidation method (Pujo-Pay & Raimbault, 1994; Raimbault *et al.*, 1999a; 1999b).

Samples for the heavy metal analysis were collected from a total of 21 stations subjected to chemical monitoring according to the network requirements. Dissolved Cd, Ni, Pb (priority substances according to the WFD as well as Co, Cu, Fe, Mn and Cr) were analyzed in the surface and bottom layers (42 samples). The analyses of Cd, Ni, Pb, Cu, Fe, and Mn were conducted according to Willie *et al.* (1998) and Milne *et al.* (2010). The Cr was analyzed according to the method proposed by Cranston & Murray (1978). Metal determinations were conducted using an ICP-MS (Thermo Element Xseries II).

### *Biological quality*

The biological quality is determined by the quality of the basic biological elements used also by the WFD. Classification of the biological quality elements was based on the results of the Mediterranean ecoregion in-

tercalibration exercise (GIG, 2013) using the intercalibrated methodologies for Greece and the Eastern Mediterranean, namely the Bentix and the EEIc. However, in order to be compatible for the environmental status assessment within the MSFD, the classification tool for the benthic macroinvertebrates was extended to include a combination of the Bentix Index and the diversity parameters (Simboura *et al.*, 2012; 2014) necessary for the assessment of the environmental status in relation to the condition of the benthic communities.

For each biological quality, the metric values were expressed as a standard unit termed the Ecological Quality Ratio or EQR. The Ecological Quality Ratio (EQR) ranges from 0 to 1 and is used as the standardized measure for correlations among the various indices. For each ecological quality metric in a given sampling site, this EQR value results from the ratio of the value observed versus the value of the same metric under the reference conditions.

### **Phytoplankton-eutrophication**

The biomass of the phytoplankton (as chlorophyll- $\alpha$ ) constitutes an important ecological quality descriptor for the coastal environment. The phytoplankton biomass is most frequently measured as the concentration of chlorophyll- $\alpha$  at discrete depths in the euphotic portion of the water column and is measured in the seawater samples collected also for the physicochemical parameters from the discrete standard depths of 2m, 10m, 20m, 50m and the bottom layer. Chlorophyll- $\alpha$  was measured in the laboratory with a fluorometer, TURNER 00-AU-10, according to Holm-Hansen *et al.* (1965). All the phytoplankton biomass values were integrated over depth according to the trapezoid rule (Culley & Welschmeyer, 2002). The estimation of the ecological quality status of the phytoplankton was based on the mean integrated values of chlorophyll- $\alpha$ , according to the scale suggested by Karydis (1999); Ignatiades *et al.* (1992); Pagou *et al.* (2002) and Simboura *et al.* (2005). The classification scale of the chlorophyll- $\alpha$  concentrations has been proposed by Karydis (1999) and used extensively for the Greek seas. This scale includes four eutrophication levels, viz., the eutrophic, higher mesotrophic, lower mesotrophic and oligotrophic, and was later modified by Simboura *et al.* (2005) to comply with the five levels of the ecological status of the Water Framework Directive. The boundaries of this scale were intercalibrated during the WFD Intercalibration activity. Although the composition of phytoplankton is one of the mandatory WFD parameters for phytoplankton, it was not intercalibrated within the Mediterranean ecoregion and, therefore, not included here.

Moreover, for assessing the eutrophication status, the Eutrophication Index (EI) of Primpas *et al.* (2010) was used, combining the concentrations of the nutrients (phosphate, nitrate, nitrite, ammonia) and the chlorophyll- $\alpha$  biomass into a single formula to produce a five-scale scheme according to the WFD requirements:

$$E.I.=0.279C_{PO4} + 0.261C_{NO3} + 0.296C_{NO2} + 0.275C_{NH3} + 0.214C_{Chl-\alpha}$$

(High) less than 0.04; (Good) 0.04-0.38; (moderate) 0.38-0.85; (poor) 0.85-1.51; (bad) >1.51

According to the Eutrophication Index ranges reported by Primpas *et al.* (2010), oligotrophy corresponds to the ranges of EI (0.04-0.38), mesotrophy to the EI range (0.37-0.87) and eutrophication to EI (0.83-1.51). The upper limit of the moderate range of the EI scale was set as the average of the lower limit of the eutrophic and the upper limit of the mesotrophic groups.

### **Macroalgae**

Macroalgae were collected from the representative rocky shores in the water bodies checked by free diving along the upper rocky infralittoral zone, from the almost horizontal surfaces, 30–50 cm below the lowest water level. Macroalgae within one quadrat of 400 cm<sup>2</sup> (20 × 20 cm), considered to be the minimal representative sampling surface for the Mediterranean infralittoral communities (Dhont & Copejans, 1977; Boudouresque & Belsher, 1979), were scraped off with a chisel in each station. The material collected was preserved in buffered 4% formalin seawater. In this work, the results from the 31 stations sampled for macroalgae will be presented.

In the laboratory, the species were identified down to the lowest possible taxonomic level, and the abundance of each taxon was estimated as the percent coverage in the sampling surface in the horizontal projection (Verlaque, 1987). The cumulative coverage of the taxa per sampling unit would often exceed 100% due to the multiple layers of vegetation (canopy, bushy, epiphytes).

The Ecological Evaluation Index (EEI) by Orfanidis *et al.* (2001; 2003) and its continuous values version EEIc (Orfanidis *et al.*, 2011) were used for the ecological evaluation of the biological element of the macroalgae.

### **Benthic Macroinvertebrates**

Benthic samples were collected from each station using a Smith McIntyre grab over a 0.1m<sup>2</sup> sampling surface. Two replicate samples were collected in each station from the soft bottom sediments. Samples were sieved on board through a 1mm mesh size sieve, preserved in 4% formalin solution in water and sorted out in the laboratory. The benthic species were identified to the species level wherever possible. For the assessment of the ecological status (WFD) the intercalibrated Bentix Index is used; however, for the MSFD assessment of benthic communities, diversity parameters also need to be taken into account (EC, 2010).

To this end the method of Simboura *et al.*, (2012; 2014) was applied by integrating the indicators for the condition of benthic communities under the Sea-floor Integrity descriptor of MSFD and proposing thresholds and reference values for Good Environmental Status (GENS)



for the Eastern Mediterranean for Diversity indices.

This method is an adaptation of a similar method applied across the MSFD descriptors in the Basque country (Borja *et al.*, 2011). The scheme proposed is a modular formula assigning weighting scores to each one of the components of a formula: one 'biotic' component, two 'diversity' components and one 'size' component. The size component was not included here as size spectra analysis for benthic communities is a labor-intensive process and such data are difficult to obtain and are unavailable for this dataset.

Each component in the formula is expressed by the EQR, weighted accordingly and the sum of all the weighted values corresponds to the final Environmental Status. The weighting scores were selected taking into account that a) the Ecological Quality Status (EQS) within the WFD and the Environmental Status (ES) within the MSFD should be harmonized and the two Directives should be fully and seamlessly integrated (Borja *et al.*, 2010); b) the conclusion that at least in the Mediterranean Sea the Shannon diversity shows a non-monotonic response to the pressure gradients and that the biotic indices are more efficient to assess the EQS (Subida *et al.*, 2012); c) the species richness is a highly variable indicator and shows a less significant correlation with the EQS than the Shannon index (Simboura *et al.*, 2012).

The derived formula is as follows:

$$\text{Bentix EQR} \times 0.6 + \text{Shannon EQR} \times 0.2 + \text{Species richness EQR} \times 0.2 = \text{ES}$$

The final Environmental status (ES) is expressed as an EQR value and classified according to the standard scale: 1=high; 0.8=good; 0.6=moderate; 0.4=poor; 0=bad.

For naturally stressed biotopes such as the coastal areas close to the Evrotas and Kalamas, Louros and Arachthos

river mouths the scale used for assessing the environmental status was not the standard one but the one derived from the European intercalibration (Borja *et al.*, 2007; 2009), viz., 0.77, 0.53, 0.38, 0.20, 0 and used by other authors for the classification of estuaries (Borja & Tunberg, 2011). The Bentix EQR used in the multimetric formula is normalized according to the scale used for determining the final ES.

For the calculation of the Bentix Index (Simboura & Zenetos, 2002) an Add-in v.1.0 version software for MS Excel 2007 has been used, downloaded free from: <http://www.hcmr.gr/en/>.

For the calculation of H and S EQRs the reference values for each ecotype (Simboura *et al.*, 2012) were set as follows: ecotype A (coastal muddy) and C (deeper than 90m, muddy): S=40 and H=5; and for ecotype B (coastal, mixed): S=100, H=6. These values refer to the standard sampling surface for the benthos (0,1m<sup>2</sup>) (UNEP/MAP, 2004). These reference values were derived from the maximum values encountered over the Hellenic seas (Simboura *et al.*, 2012; UNEP/MAP, 2004). Specifically, for three stations at depths greater than 90m but with unclear ecotype classification the reference values were set as follows: S=21 and H=4 whereas the Shannon reference values correspond to the maximum possible diversity for the maximum richness (21 species) found in these data calculated as:  $H_{\max} = \log_2(S_{\max})$ .

### Integrative environmental status

In the present work, a modification of the decision tree presented by Borja *et al.* (2004; 2009a) will be used for an integrative assessment of the environmental status within the Marine Strategy Framework Directive (Fig. 1).

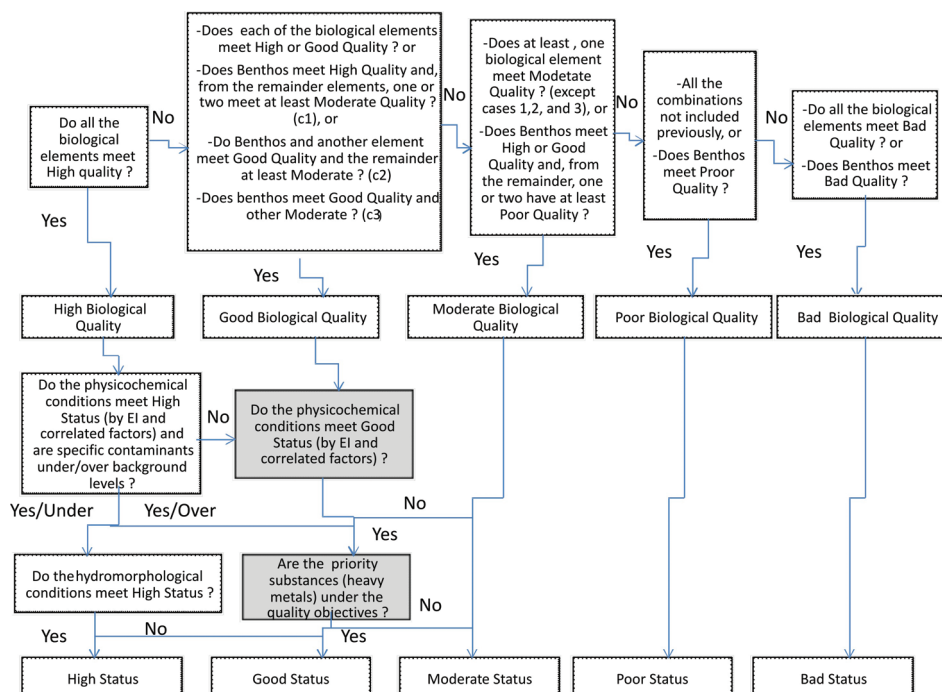


Fig. 1: Decision tree modified from Borja *et al.* (2004; 2009a) for integrative ecological status assessment.

The modification lies in the representation of the general physicochemical status by the Eutrophication Index (EI) of Pimpas *et al.* (2010), an index developed for the Eastern Mediterranean oligotrophic waters combining the nitrates, nitrites, ammonium and phosphates together with the chlorophyll-*a* biomass levels. The oxygen values and transparency through the Secchi disk disappearance depth were also considered in the evaluation of the physicochemical conditions. It should be highlighted that the chlorophyll-*a* is used separately as an evaluation measurement of the biological quality element of the phytoplankton. Correlation of the EI index with the physicochemical parameters will assess the representativeness of this index as a 'general physicochemical status' descriptor.

The biological quality was assessed based on the three biological quality elements, following the decision tree giving special weightage to the benthic communities, which are good indicators of the quality of the whole ecosystem due to their limited mobility, and for which also the mature and intercalibrated methodologies tested for the different pressures are available as recommended (Borja *et al.*, 2009a; Prins *et al.*, 2013). It is noted here that in the original decision tree, case 3 (c3) is intended only for coastal areas which are however exclusively treated in this work. In cases where the biological quality is 'moderate', 'poor' or 'bad', the corresponding ecological status is 'moderate', 'poor' or 'bad,' respectively. In cases where the biological quality is 'high' or 'good', a series of steps involving the hydromorphological, the physicochemical conditions and the priority substances/specific contaminants are followed (Borja *et al.*, 2004).

Among the priority substances, only the heavy metal concentrations are available. Their concentrations were evaluated with respect to the quality standards defined by the national and EU regulations (EC, 2008b).

It should be highlighted that the decision tree described, using the Bentix index as a classification metric of the benthic communities, was applied as an integrative assessment method for the implementation of the WFD in the Grecian coastal waters (HCMR, 2012).

To compare the classification given by the different tools used in relation to the final integrative classification, a weighted Kappa analysis was undertaken (Cohen, 1960; Landis & Koch, 1977) applying the methodology presented by Borja *et al.* (2007). This analysis takes into account that the importance of misclassification is not the same among the close categories (for example, high or good, moderate or poor) as between further categories (for example, between high or good and moderate or poor). Disagreements involving the categories apart from the threshold of degraded-undegraded status are referred to as the percentage of mismatch or problem cases. The kappa values reveal the next levels of agreement: (i) Null < 0.05; (ii) Very low: 0.05–0.2; (iii) Low: 0.2–0.4; (iv) Moderate: 0.4–0.55; (v) Good: 0.55–0.7; (vi) Very Good: 0.7–0.85; (vii) Almost perfect: 0.85–0.99; and (viii) Perfect: 1 (Monserud & Leemans, 1992).

As a method for integrating results at the water body level (Fig.10), the method of "worst case" or the OAOA rule was used.

### **Pressure and State of Change indicators**

The pressure index derived from Borja *et al.* (2011) was estimated for the water bodies monitored to give the magnitude of the anthropogenic pressures imposed (Table 1). According to this methodology, a pressure intensity scale is defined from 3 (high) to 1 (low) providing values for each pressure type within the corresponding area. The pressure index is calculated as the average pressure intensity. The classification of the pressures was based on the Water Information System for the Europe (WISE-SoE) reporting system for the coastal and marine waters (<http://cdr.eionet.europa.eu/gr/eea>) and data available through the Article 5 implementation of WFD, in Greece (HCMR, 2008, [www.minenv.gr](http://www.minenv.gr)). The pressure types include sewage discharge, industrial discharge, other discharges, spoiled waste, other waste, mariculture, fishing, marinas, ports, and other activities.

The integrated (over the whole water column) values of Total Carbon (TC), Total Nitrogen (TN), Particulate Organic Carbon (POC) and Particulate Organic Phosphorus (POP), dissolved oxygen and bottom layer dissolved oxygen, the transparency and organic carbon and nitrogen content in the sediment were used as environmental indicators of the state of change, following the European Environmental Agency (EEA) Drivers, Pressure, State, Impact, Response (DPSIR) approach. Correlations of these environmental indicators with the integrative ecological status were made.

For substituting status class with the parametric values in the correlations of the integrative status with the environmental and pressure parameters, an equivalence value was used according to the one proposed by Borja *et al.* (2009a). For high status the equivalence value is 10, for good 8, for moderate 6, for poor 4 and for bad 2.

The STATGRAPHICS CENTURION 2009 software package, StatPoint Technologies, Inc., was used to explore statistically significant differences or correlations across the biotic and environmental parameters. For testing correlations, the Pearson correlation coefficient was calculated.

## **Results**

### **Hydromorphological conditions**

The speed of the coastal currents recorded was in general weak, i.e., in the range of 5-10 cm/sec, in the upper 15 m. In the deeper layers it was even less, in the order of 2-3 cm/sec. This occurred mostly in the isolated areas that had minimal exposure to the open sea. Higher speeds, near 15-20 cm/sec were recorded near Korinthos and in the Thessaloniki Gulf (TP16, TP10), most likely

**Table 1.** Indices values and Environmental Quality Status (EQS) assessment of the biological quality indices, eutrophication status, biological status and integrative environmental status. Pressure index value. H=high, G=good, M=moderate, P=poor, B=bad, NA=not available.

STATION	Benthic-H-S EQS	Benthic-H-S EQS	EEIC	EEIC EQS	Chl-a	Chl-a EQS	EI	Eutroph. status	Biolog. EQS	Integr. ES	Pressure index
Pylos	0,51	M	3,91	P	0,337	G	0,193	G	M	M	1,1
Methoni	0,69	G	5,03	M	0,205	G	0,197	G	G	G	1,1
Korinthos	0,66	G	3,87	P	0,299	G	0,320	G	M	M	1,1
Aigio	0,70	G	6,02	G	0,486	M	1,175	P	G	M	1,1
Xylokastro	0,79	G	5,52	M	0,608	P	0,808	M	M	M	1,1
W. Patraikos	0,68	G	7,59	G	0,156	G	0,609	M	G	M	1
Patra	0,78	G	7,22	G	0,314	G	0,870	P	G	M	1,3
S. Patraikos	0,55	M	4,46	M	0,238	G	0,404	M	M	M	1
Argostoli	0,80	G	4,08	M	0,666	P	0,333	G	M	M	1,2
Argolikos	0,63	G	6,96	G	0,416	M	0,535	M	M	M	1,2
Vourlias	0,74	G	9,20	H	0,279	G	0,312	G	G	G	1,1
Evrotas	0,60	G	NA	NA	0,296	G	0,228	G	G	G	1
Messolonghi	0,60	M	9,60	H	0,262	G	0,819	M	G	M	1,2
South Amvrakikos	0,29	P	2,00	B	0,860	P	1,228	P	P	P	1,6
Igoumenitsa	0,47	M	9,64	H	1,324	P	0,508	M	M	M	1,4
Kalamas	0,58	G	8,39	H	0,288	G	0,278	M	G	M	1,2
Ekvoles Lourou	0,51	M	2,00	B	2,572	B	1,069	P	M	M	1,4
Ekvoles Arachthou	0,32	P	NA	NA	1,860	P	0,880	P	P	P	1,4
South Evvoikos	0,61	G	NA	NA	0,127	G	0,173	G	G	G	1,3
S1	0,26	P	5,83	M	1,398	P	0,489	M	P	P	1,4
S8	0,44	M	4,52	M	0,154	G	0,169	G	M	M	1,2
S11	0,56	M	5,37	M	0,153	G	0,122	G	M	M	1,2
S7	0,51	M	5,18	M	0,194	G	0,381	M	M	M	1,4
Faneromeni	0,47	M	NA	NA	0,577	M	0,223	G	M	M	1,1
S25	0,30	M	5,37	M	0,170	G	0,285	G	M	M	1,1
Maliakos	0,72	G	2,00	B	1,002	P	0,417	M	M	M	1,3
Asopos	0,46	M	NA	NA	0,363	G	0,250	G	M	M	1,2
Itea	0,53	M	NA	NA	0,746	P	0,843	M	M	M	1,3
Antikyra	0,73	G	6,81	G	0,680	P	0,522	M	G	M	1,2
Theologos	0,64	G	5,57	M	0,235	G	0,615	M	M	M	1,1
Skouries	0,44	M	NA	NA	0,281	G	0,568	M	M	M	1,4
Larymna	0,54	M	NA	NA	1,106	P	0,581	M	M	M	1,5
Volos	0,64	G	4,70	M	0,166	G	0,137	G	G	G	1,2
TP32	0,52	M	NA	NA	0,615	P	0,301	G	M	M	1,2
Stratoni	0,36	P	10,00	H	0,201	G	0,152	G	P	P	1,2
TP16	0,47	M	3,06	P	2,232	B	0,685	M	M	M	1,2
TP10	0,60	M	3,81	P	1,769	P	0,492	M	M	M	1,6
KAV3	0,53	M	NA	NA	0,638	P	0,384	M	M	M	1,6
Diavlos Thasou	0,66	G	NA	NA	0,355	G	0,143	G	G	G	1,2
IG2	0,64	G	6,16	G	0,156	G	0,169	G	G	G	1,4
Souda	0,62	G	6,85	G	0,128	G	0,219	G	G	G	1,4
Messara	0,71	G	6,05	G	0,059	H	0,067	G	G	G	1
Moudros	0,64	G	NA	NA	0,299	G	0,113	G	G	G	1
Limnos	0,90	H	NA	NA	0,049	H	0,104	G	H	H	1

related to the tidal influence. The general hydromorphological conditions were normal corresponding to the salinity and density of the Eastern Mediterranean type of coastal waters 'not influenced by freshwater input'.

### **Physicochemical conditions**

The temperature and salinity CTD measurements, during the period of April and May 2012, showed the typical values of these parameters found in the coastal marine environment around Greece for this period (Kontoyiannis *et al.*, 2005). Temperatures ranged approximately from 16 to 19°C, in the surface layers and between the 15-11°C near the bottom. Isolated water bodies such as the gulfs and the narrow passages exhibited comparatively lower surface temperatures in April and higher in May. The surface salinity values ranged from the minima of ~26-28, near the river mouths of the Amvrakikos Gulf, to ~35-36 in the North Aegean areas and to the maxima of ~38.5-39.1 in the South Aegean. Higher salinity values existed in the subsurface and near-bottom layers in almost all the stations. These values ranged from ~36-38.5 in western coastal Greece to 38.5-39.3 in the Aegean.

The Secchi disk transparency values ranged from 2.5m to 22m. Lower values were recorded near the river-mouths, especially those in western Greece (Amvrakikos Gulf), and in the enclosed bays or protected gulfs (Argolikos Gulf, Larymna Bay, Maliakos Gulf, Thessaloniki Gulf, Elefsis Bay, Faneromeni Bay). The southern Aegean and the areas near and around Crete were characterized by the highest transparency values (19-22 m), whereas the area of the Inner Saronikos Gulf (stations S7, S8, S11) had Secchi-disk transparency values around ~15-20 m.

In general, the waters of the Greek coastal areas are well oxygenated. Relatively lower dissolved oxygen (DO) DO values (4.25mL/L) were recorded near the bottom of Elefsis Bay. In Elefsis Bay, the development of a strong temperature-driven pycnocline during the period of May-November every year, results in the isolation of the deeper part of the water column, leading to insufficient oxygen supply from either the atmospheric or photosynthetic sources and the significant decrease in the oxygen content, resulted in the anoxic conditions during the summer period. In addition, low DO concentrations (<2.00 mL/L) were recorded near the bottom of the Amvrakikos Gulf due to the consumption of the high organic matter content discharged from the rivers.

Nutrient concentrations during April and May 2012 revealed significant spatial variation among the various coastal areas of Greece influenced by different points and/or diffuse anthropogenic pressures (related to nutrient enrichment).

The nutrient values recorded in the coastal zones of Greece were highly deviated from the average value and ranged as follows: Nitrate: < LOQ (limit of quantification)–5.100 µmol/L (0.634±0.025 µmol/L); nitrite: < LOQ–0.579

µmol/L (0.106±0.096 µmol/L); silicate: 0.85 µmol/L – 41.7 µmol/L (4.65±7.67 µmol/L); ammonium: 0.051 – 2.994 µmol/L (0,385±0,402 µmol/L); DIN:P ratio (referring to the Dissolved Inorganic Nitrogen: nitrate+nitrite+ammonium ratio to inorganic phosphorus): 1-98 (29±23).

The highest mean integrated nitrate concentrations were recorded in Korinthiakos Gulf, Patraikos Gulf and North Evvoikos Gulf influenced mainly by industrial activities, as well as in the Amvrakikos Gulf which is enriched in nutrients through the riverine outfalls from the Arachthos and Louros rivers. The mean integrated Ammonium concentrations were lower than 0.50 µmol/L in most parts of the Greek coastal areas, whereas relatively higher ammonium mean integrated values were recorded in the areas affected by industrial activities. It is noteworthy, that a relatively high mean integrated value of ammonium (1.13 µmol/l) was recorded in the Louros estuary, indicating pollution problems in the river.

The Amvrakikos Gulf together with Patras port and Pyttalia sewage outfall were enriched in phosphate. Consequently, the DIN:P ratio was calculated below the theoretical value (16:1) (Redfield,1958) indicating nitrogen limitation. Thus, the Saronikos Gulf, Thermaikos Gulf and Amvrakikos Gulf seem to be nitrogen limited whereas, the rest of the coastal Greek areas were phosphorus limited (Fig. 2). The Amvrakikos Gulf was also highly enriched in silicate (34 µmol/L in the south Amvrakikos Gulf and 26 µmol/L in the north Amvrakikos Gulf).

The DIN:Si ratio was below 1 in all the coastal areas studied in Greece suggesting a higher silicate concentration relative to inorganic nitrogen (Fig.3). The ratio of silicate to inorganic phosphorus (Si:P) was extremely high, indicating a strong deficiency of P relative to Si for the Greek coastal zones (Fig. 4).

The Eutrophication Index (EI) of Pimpas *et al.* (2010) was used to assess the combined effect of all the nutrients on the physicochemical status. Fig. 5 shows the Eutrophication Index values (EI) in each station. The EI recorded three classes of water quality, namely, good, moderate and poor. The physicochemical status was judged as good in stations where the eutrophication index rendered good status and also where the oxygen values and Secchi disk disappearance depth were not ranging among the lower values.

### **Metals**

Dissolved Cd, Ni and Pb concentrations were found below the environmental quality standards set by the Directive 2008/105/EC (EC 2008b) by one order of magnitude for Cd and Pb and two orders of magnitude for Ni. Cadmium ranged from 0.005 to 0.21µg L<sup>-1</sup> (0.012 ±0.004µg L<sup>-1</sup>); Pb from 0.113 to 2.55µg L<sup>-1</sup> (0.377±0.345µg L<sup>-1</sup>); Ni from 0.277 to 1.55µg L<sup>-1</sup> (0.620±0.269µg L<sup>-1</sup>). The corresponding quality standard values are 0.2µg L<sup>-1</sup> for Cd, 20µg L<sup>-1</sup> for Pb and 7.2µg L<sup>-1</sup> for Ni. Metals not included in the priority substances



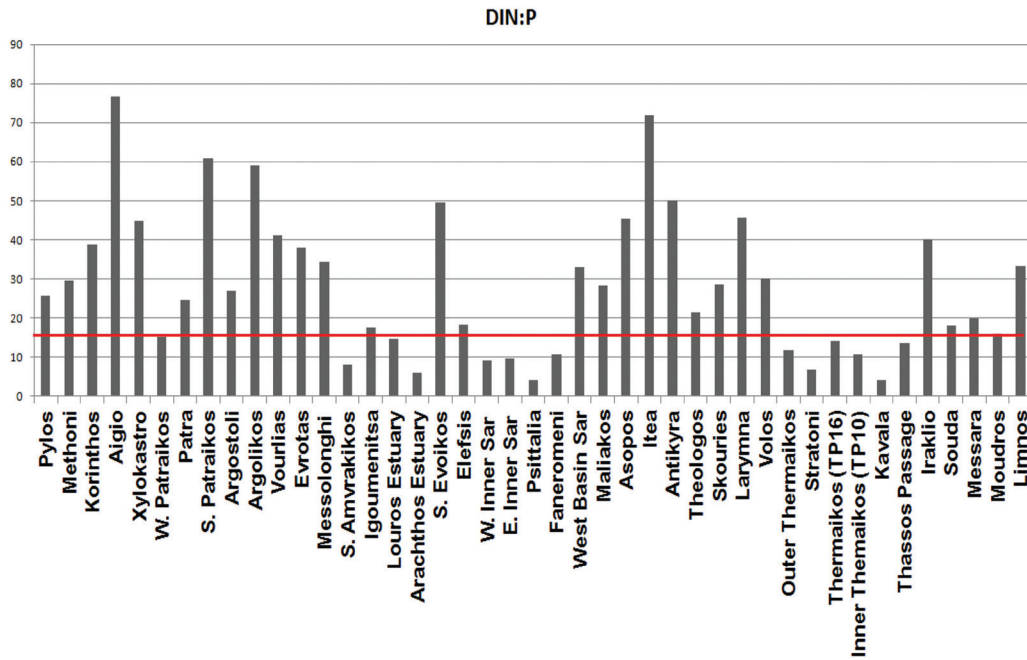


Fig. 2: Mean integrated DIN:P ratios in the various coastal areas of Greece during April-May 2012. The line shows the theoretical value of 16:1.

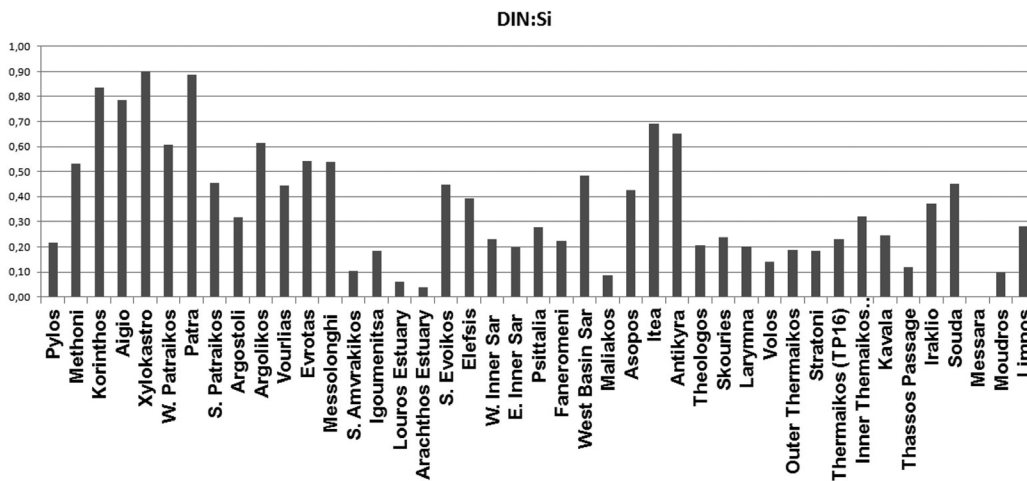


Fig. 3: Mean integrated DIN:Si ratios in the various coastal areas of Greece during April-May 2012.

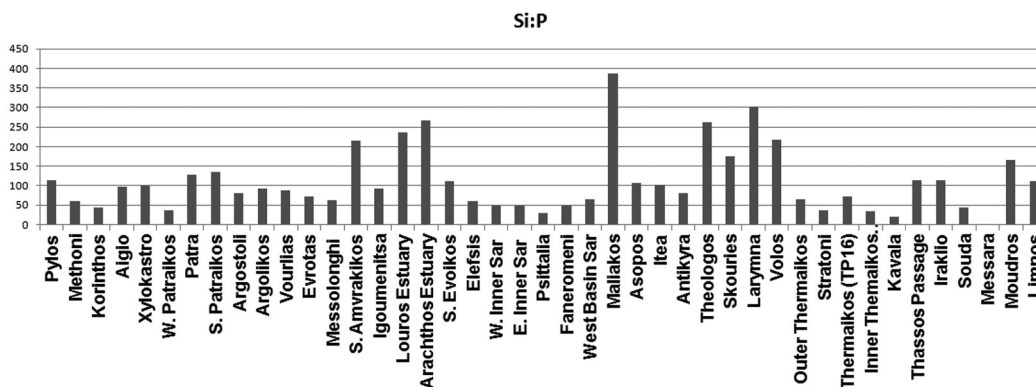


Fig. 4: Mean integrated Si:P ratios in the various coastal areas of Greece during April-May 2012.

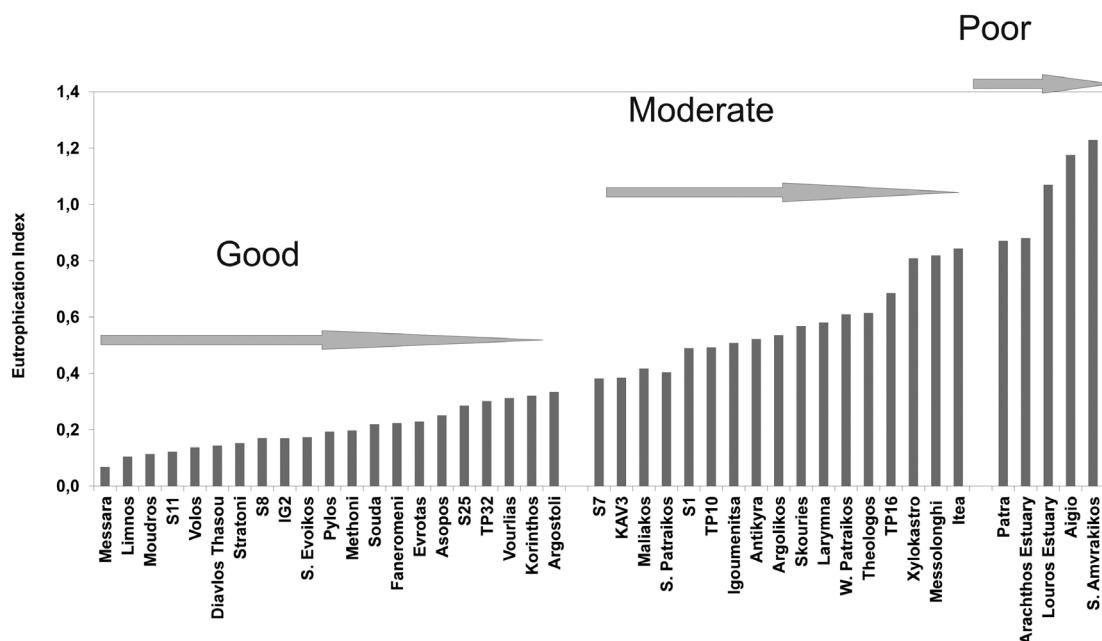


Fig. 5: Gradient of the Eutrophication Index (EI) values and classification under good, moderate and poor ecological status.

list (Co, Cu, Zn, Cr) are considered specific pollutants; however, quality standards have not been established for the coastal waters. The concentration ranges recorded for these metals during our survey are as follows: Co:  $0.005\text{--}0.021\mu\text{g L}^{-1}$  ( $0.048\pm 0.025\mu\text{g L}^{-1}$ ); Cu:  $0.149\text{--}2.16\mu\text{g L}^{-1}$  ( $0.339\pm 0.267\mu\text{g L}^{-1}$ ); Zn:  $0.641\text{--}4.28\mu\text{g L}^{-1}$  ( $1.79\pm 0.774\mu\text{g L}^{-1}$ ); Cr:  $0.269\text{--}0.815\mu\text{g L}^{-1}$  ( $0.475\pm 0.109\mu\text{g L}^{-1}$ ). Overall, these values do not show important pollution loads. Occasionally, however, for some samples, increased metal concentrations were recorded, shown as the outlier values in the box-whisker diagram of Fig. 6. It is noteworthy that 35% of the outlier values correspond to the metal concentrations measured in station S1 in the Elefsis Bay where the largest industrial zone of Greece is located.

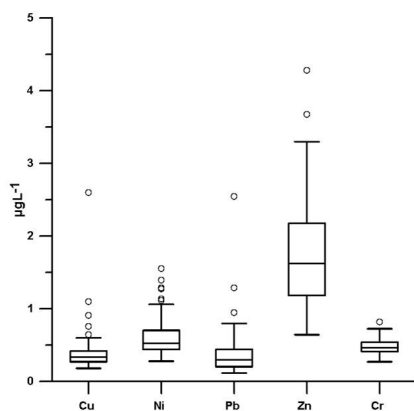


Fig. 6: Box-whisker diagrams for Cu, Ni, Pb, Zn, Cr ( $\mu\text{g L}^{-1}$ ) concentrations measured during 2012 samplings in the Greek coastal waters. Data for Cd and Co not shown.

## Biological quality

### Chlorophyll-*a*

Figure 7 presents the gradient of the mean integrated values of chlorophyll concentration. It is obvious that the highest standing stock of chlorophyll-*a* was recorded in the Inner Thermaikos Gulf ( $2.232\pm\text{mg m}^{-3}$ ), in the Louros and Arachthos Estuaries (Amvrakikos Gulf) with concentrations of  $2.572\pm 0.09\text{mg m}^{-3}$  and  $1.860\pm 0.35\text{mg m}^{-3}$ , respectively, and in Elefsis Bay with a chlorophyll value of  $1.398\pm 0.76\text{mg m}^{-3}$  (Saronikos Gulf). Low chlorophyll concentrations were recorded in two stations situated in Limnos (North Aegean Sea) and Messara (Crete) with mean integrated values of  $0.049\pm 0.01\text{mg m}^{-3}$  and  $0.059\pm 0.01\text{mg m}^{-3}$ , corresponding to the high ecological quality.

Areas assessed as having moderate status include sites in the Argolikos Gulf, in Aigio (northern coast of Peloponnese) and Faneromeni Bay (Saronikos Gulf). The majority of the areas sampled during April and May 2012 were assessed as being in good status and are situated in Evvoikos Gulf, Argolikos Gulf, outer Thermaikos Gulf, southern coasts of Peloponnese and in the Western Basin of the Saronikos Gulf.

High chlorophyll-*a* values corresponding to bad ecological quality were noted in the North Amvrakikos Gulf, which is influenced by riverine outflow (Arachthos and Louros rivers) and in the Inner Thermaikos Gulf area influenced by the anthropogenic nutrient enrichment and industrial activities.

The POC concentrations measured exhibited high spatial as well as vertical variability. The POC values during the April and May 2012 sampling ranged between  $4.4$  and  $33.1\mu\text{molC L}^{-1}$  (mean $\pm$ stdev:  $11.0\pm 6.5\mu\text{molC L}^{-1}$ ). The

highest mean depth-integrated POC concentrations that were markedly higher than those recorded in the other water bodies were observed in the Elefsis Bay area characterized by strong industrial activity (e.g. oil refineries, steelworks, and shipyards) and the Gulf of Thessaloniki – a region influenced by riverine inputs and sewage effluents. The water bodies of Faneromeni Bay, Gulf of Avlis, Larymna Bay, Maliakos Gulf and Inner Thermaikos Gulf appear moderately enriched in organic particles.

#### *Benthic macroinvertebrates*

On the whole, 4852 benthic macroinvertebrate individuals were counted and 510 species identified. The Bentix-H-S combination multimetric tool used as a classification metric for the benthic macroinvertebrates corresponded (Table 1) to poor ecological quality in three cases: in Elefsis Bay, Amvrakikos Gulf station and Ierissos Gulf (Stratoni station). Moderate status was noted in several sites: the southern Patraikos Gulf, Itea Bay at Korinthiakos Gulf, North Evvoikos Gulf (Skouries), Gulf of Avlis, and Kavala Gulf, all being water bodies influenced mainly by industrial and other activities. Other water bodies classified by the combined Bentix-H-S tool as in moderate status are the Thermaikos Gulf and Inner Saronikos Gulf, which are mainly influenced by treated sewage effluents and eutrophication.

In the western basin of the Saronikos Gulf, the low number of benthic species found (4 species and 14 specimens) was below the confidence level of the index to allow a reliable classification by the Bentix Index. However, considering the specific hydro-morphological conditions prevailing in this deep trench-shaped and hypoxic basin which acts as a sink for nutrients and organic matter (Frigolis, 1985), the condition of the sparse benthic communities of the area was characterized as moderate. Pylos Bay, an enclosure of the Ionian Sea and Kalamas station, influenced by riverine outflow, was also classified as having a moderate status by the Bentix Index. Good ecological quality was shown by the Bentix-H-S tool in 20 stations and high ecological quality at one station in Limnos Island (North Aegean).

#### *Macroalgae*

The lowest values on the Ecological Evaluation Index (EEIc) applied were observed in the South Amvrakikos, Louros estuary, Maliakos Gulf, Inner Thermaikos and Thessaloniki Gulf. Poor status was assessed for the Pylos and Korinthos stations. Stations assessed as having moderate status include Methoni, Xylokastro, S. Patraikos, Argostoli, stations of the Inner and Western Saronikos Gulf, Elefsis Bay, Faneromeni Bay, North Evvoikos Gulf and Volos. Good status was found in Aigio, W. Patraikos and Patra, Argolikos, Antikyra Bay, and in all stations of Crete (IG2, Souda, Messara), while high status characterized Vourlias, Messolonghi, Igoumenitsa Bay, Kalamas and

Stratoni. In some stations a rocky substrate was absent and macroalgae were not sampled (marked as NA in Table 1).

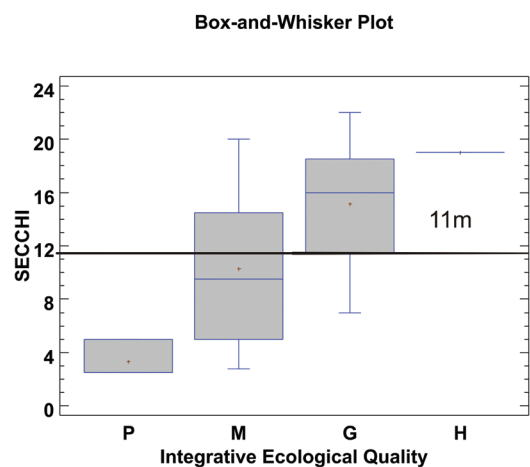
#### *Integrative ecological status and validation*

Table 1 shows the pressure index values, results of the biological quality indices, eutrophication index, derived classification status, biological quality classification and integrative or global status classification following the decision tree (Fig. 1) as modified from Borja *et al.* (2004). Analytical data on the values of the various indices used (Bentix, Shannon, species richness) and sampling station characteristics (coordinates, depth, substrate) are given in the Annex (as Supplementary Data, only on line) on request by the corresponding author.

The box-plot of Fig. 8 shows the plotting of the Secchi disk disappearance depth against the integrative status classes. The differences in the Secchi disk values among the classes are statistically significant (F-Ratio=4,68 and P-Value=0,0068) and the lower value of the lower quartile of the box-plot of the data is 11m depth. This limit could be set as a tentative threshold value for good status for use in the decision tree along with the results of the Eutrophication Index to assess the physicochemical status.

Figure 9 (a-f) shows the assessment of each classification tool used separately at the level of the monitoring stations highlighting on the pie-graphs the percentage of the different classes provided by each tool. The results of the class agreement analysis in relation to the integrative classification, showed that the multimetric tool and Bentix demonstrated a very good (kappa value=0,72, 22,7% mismatch cases) and good (kappa value=0,60, 25% mismatch cases) agreement, respectively.

The multimetric tool displays a better agreement with the integrative status compared with the Bentix, as it was used as a tool for the benthic element in the decision tree. The Eutrophication Index (kappa value=0,54, 22,7% mismatch cases) and chlorophyll-*a* (kappa value=0,45, 34,1% mis-



**Fig. 8:** Box-whisker plot of the Secchi disk disappearance depth across the integrative ecological quality classes. Good to moderate threshold limit located at 11m depth.

**Table 2.** Results of the coastline length and area GIS classification based on a) 2012 monitoring results b) 2008 baseline monitoring results of the respective water bodies (WB) and c) 2008 baseline monitoring results of the whole country.

Ecological quality status (EQS) classification	a) monitoring 2012 - integrative classification				
	count of WBs per category	coastline - length (km)	% length coastline	area (km <sup>2</sup> )	% area
High	18	1601,18	32,89	4152,54	25,41
Good	8	410,46	8,43	1259,08	7,71
Moderate	33	2533,35	52,04	10441,47	63,90
Poor	3	323,01	6,64	486,37	2,98
Sum	62	4867,99	100,00	16339,46	100,00
<b>b) 2008 baseline monitoring results of same water bodies</b>					
EQS classification	count of WBs per category	coastline - length (km)	% length coastline	area (km <sup>2</sup> )	% area
High	21	1781,12	36,59	4867,99	28,64
Good	12	888,03	18,24	5451,88	33,37
Moderate	26	2048,80	42,09	6061,11	37,09
Poor	3	150,05	3,08	147,15	0,90
Sum	62	4867,99	100,00	16339,46	100,00
average class % deviation 2008-2012			6,20		14,04
<b>c) 2008 baseline monitoring -whole country</b>					
EQS classification	count of WBs per category	coastline - length (km)	% length coastline	area (km <sup>2</sup> )	% area
High	155	11006,32	65,22	19623,27	51,12
Good	40	3002,40	17,79	11041,39	28,76
Moderate	35	2717,69	16,10	7578,03	19,74
Poor	3	150,05	0,89	147,15	0,38
Sum	233	16876,46	100,00	38389,83	100,00

match cases) showed a moderate class agreement, whereas the macroalgae index showed a low agreement (kappa value=0,27, 32,3% mismatch cases) with the integrative status. The Eutrophication Index had a low class agreement with both the multimetric and Bentix benthic indices.

Figure 10 shows the Arc GIS mapping of the classified water bodies of Greece based on the first year monitoring results. Table 2 shows a) the GIS calculation of the coastline length and area classification in each ecological status class of the 62 water bodies evaluated during 2012, b) the baseline classification (HCMR, 2008) for case (a) stations and c) the baseline classification of the whole country.

Table 2 also shows the calculated average percentage deviation between the 2012 classification results and the 2008 baseline classification results, which is 6,2% for the coastline length and 14 % for the area classification. It is then deduced that the eventual classification of the whole country coastline may be close (with a deviation of 6%) to the one given under the baseline classification with a percentage of 65,22% in the high class, 17,79% in the good and 16,10% in the moderate class. It is noteworthy, however, that the baseline monitoring gave a higher percentage of good and high classes than did the 2012 monitoring, overestimating the EQS.

Table 3 shows the Pearson correlation coefficient and p-values among the biotic indices, pressure index and abiotic parameters used as the state of change indicators. Chlorophyll- $\alpha$  concentrations correlated positively and significantly with the pressure index and the water parameters of total and particulate organic carbon and phosphorus, total nitrogen and with the water transparency estimated by the Secchi disk disappearance depth. Also, the chlorophyll- $\alpha$  values correlated significantly with the total organic carbon and nitrogen in the sediment.

The benthic macroinvertebrate Bentix index correlated negatively and significantly with the pressure index, total nitrogen and phosphorus, Particulate Organic Carbon (POC) and Phosphorus (POP) in water, positively with the N:P ratio and transparency and negatively with the sediment parameters.

The same correlations were also evident for the combination of the Bentix-H-S metric. The macroalgae EEIc index showed significant negative correlation with chlorophyll- $\alpha$ , biological quality and total phosphorus in the water. Biological quality derived from the three biological elements of the phytoplankton, macroinvertebrates and macroalgae following the decision tree of Borja *et al.* (2004), correlated significantly with all the



**Table 3.** Correlations among the ecological status indices and environmental and pressure factor-indices. Values represent correlation and P-value. Statistically significant correlations are marked with bold typing. The strongest correlations are underlined. DIN:P = dissolved inorganic nitrogen to phosphorus ratio; TN= total nitrogen in water; TP= total phosphorus in the water, POP=the particulate organic phosphorus, POC=the particulate organic carbon, O<sub>2</sub>=dissolved oxygen, bottom O<sub>2</sub>=dissolved oxygen in bottom layer, TOC=total organic carbon in sediment, N=total nitrogen in sediment, EI: the eutrophication index.

	Pressure index	DIN:P	TN	TP	POP	POC	O <sub>2</sub>	Bottom O <sub>2</sub>	Secchi Disk	TOC	N
				WATER						SEDIMENT	
Chl-a	0,4888 <b>0,0008</b>	-0,4455 0,0961	0,5923 <b>0,0004</b>	0,5598 <b>0,0011</b>	0,7538 <b>0,0000</b>	0,8489 <b>0,0000</b>	-0,4388 0,0529	-0,0222 0,9260	-0,6649 <b>0,0000</b>	0,3086 <b>0,0441</b>	0,3132 <b>0,0384</b>
BENTIX	-0,5028 <b>0,0006</b>	0,3434 <b>0,0260</b>	-0,3880 <b>0,0147</b>	-0,5208 <b>0,0007</b>	-0,4438 <b>0,0029</b>	-0,4140 <b>0,0058</b>	0,1881 0,4272	0,1881 0,4272	0,3199 <b>0,0365</b>	-0,4951 <b>0,0009</b>	-0,3441 <b>0,0239</b>
BENTIX-H-S	-0,4237 <b>0,0055</b>	0,3390 <b>0,0261</b>	-0,3988 <b>0,0238</b>	-0,5732 <b>0,0006</b>	-0,3843 <b>0,0100</b>	-0,2737 <b>0,0722</b>	0,0578 0,8033	0,2919 0,1991	0,3115 0,0395	-0,4869 <b>0,0009</b>	-0,3681 0,0140
EEIc	-0,2148 0,2459	0,1339 0,4807	-0,2761 0,2525	-0,4397 <b>0,0217</b>	0,1974 0,5847	0,1966 0,5861	0,4764 0,1640	0,4759 0,1644	0,1960 0,2906	0,1976 0,2866	-0,1666 0,3704
BIOLOGICAL EQS	-0,4839 <b>0,0009</b>	0,2990 0,0514	-0,4661 <b>0,0082</b>	-0,5967 <b>0,0004</b>	-0,4171 <b>0,0049</b>	-0,3723 <b>0,0128</b>	0,4992 <b>0,0250</b>	0,4053 <b>0,0763</b>	0,4456 <b>0,0024</b>	-0,4808 <b>0,0011</b>	-0,3906 <b>0,0088</b>
EI	0,3512 <b>0,0209</b>	0,1827 0,2409	0,7557 <b>0,0000</b>	0,8258 <b>0,0000</b>	0,3288 0,0313	0,3250 0,0334	-0,7674 <b>0,0001</b>	-0,0874 0,7142	-0,4455 <b>0,0028</b>	0,0286 0,8575	0,0599 0,7026
Integrative EQS	-0,4269 <b>0,0039</b>	0,2707 0,3291	-0,4674 <b>0,0080</b>	-0,6183 <b>0,0002</b>	-0,3433 <b>0,0225</b>	-0,3038 <b>0,0450</b>	0,5111 <b>0,0213</b>	0,5023 <b>0,0240</b>	0,4337 <b>0,0033</b>	-0,4187 <b>0,0052</b>	-0,3423 <b>0,0230</b>

components of its biological elements and also with the water and sediment parameters and pressure index. Biological quality increases with the decrease in the water nutrients, sediment organic carbon, particulate organic carbon and pressure index and with the increase in the dissolved oxygen and transparency. The Eutrophication Index showed significant positive correlations with the pressure index, total nitrogen and phosphorus in the water, particulate organic carbon and phosphorus in the water, dissolved oxygen and transparency.

The integrative global ecological status is significantly correlated with all the water and sediment parameters (including the bottom oxygen).

The strongest correlations among the abiotic or pressure factors and the biological indices were those seen among the pressure index and the Benthic index, total nitrogen and phosphorus in the water and the eutrophication index, the particulate organic carbon and phosphorus with chlorophyll- $\alpha$ , the bottom oxygen and the integrative status, transparency and chlorophyll- $\alpha$ , total organic carbon in the sediment and the Benthic index, and the total nitrogen in the sediment and biological quality. The N to P ratio correlated significantly and positively only with the Benthic and its multimetric derivative.

Chlorophyll- $\alpha$  showed the highest correlation with particulate organic carbon and phosphorus in the water and transparency. The Benthic and Benthic-H-S metric correlated best with the pressure index and total organic carbon in the sediment; the eutrophication index with total nitrogen and

phosphorus in the water and the biological quality and integrative status with the total phosphorus in the water.

Regarding comparisons among the ecological indices (Table 4), chlorophyll- $\alpha$  correlated significantly with the Benthic and the EEIc and even more strongly with the eutrophication index. The biological quality correlated significantly with the Benthic and its combined metric, the eutrophication index, the EEIc and the chlorophyll biomass. The integrative status correlated absolutely with the biological quality, followed by the Benthic multimetric, the Benthic, the EI and the chlorophyll- $\alpha$ .

## Discussion

This work presents a methodological approach (decision tree) to utilize the data acquired by the implementation of the WFD in the coastal water bodies to arrive at an integrative environmental status assessment sensu on the MSFD.

The elements acquired through the WFD monitoring address several Descriptors of the MSFD like the Biodiversity (D1) and Sea floor integrity (D6) through the benthic indices assessing the condition of the benthic communities, the eutrophication (D5) through the Eutrophication index and the chlorophyll- $\alpha$  biomass, the hydrographic conditions (D7) through the physical and hydro-morphological parameters measured and the contaminants (D8) through the heavy metals measurements.

The decision scheme applied, therefore, gives weight to the elements of biological quality and to those of the benthic macroinvertebrates, in particular, which largely

**Table 4.** Correlations among the various ecological quality indices. Values represent the correlation and P-value. Statistically significant correlations are marked with bold typing.

	BENTIX	BENTIX-H-S	EEIc	Chl- $\alpha$	EI	BIOLOGICAL EQS
EEI	0,2038	0,0199				
	0,2800	0,9153				
Chl- $\alpha$	-0,3844	-0,3295	-0,4153			
	<b>0,0109</b>	<b>0,0289</b>	<b>0,0202</b>			
EI	-0,0709	-0,2311	-0,1531	0,5250		
	0,6554	0,1360	0,4193	<b>0,0003</b>		
BIOLOGICAL EQS	0,7103	0,5974	0,3742	-0,4732	-0,7773	
	<b>0,0000</b>	<b>0,0001</b>	<b>0,0381</b>	<b>0,0012</b>	<b>0,0000</b>	
INTEGRATED EQR	0,5590	0,6453	0,1989	-0,4413	-0,5610	0,8575
	<b>0,0001</b>	<b>0,0000</b>	0,2833	<b>0,0027</b>	<b>0,0001</b>	<b>0,0000</b>

defines the integrated biological value as also proposed in an integrative methodological approach for the assessment of the environmental status for the MSFD (Borja *et al.*, 2011). This approach is in line with the Ecosystem Approach (ECAP) followed also by the Regional Sea Convention for the Mediterranean Sea (UNEP/MAP, 2012). The weighting of the macroinvertebrates was decided after the recognition (Borja & Rodríguez, 2010) that some of the biological elements and mostly the phytoplankton and macroalgae produced most instances of the disagreements (on the coastal and transitional waters) between the integrative status and the status derived by following the OAO principle of WFD. This disagreement was attributed by Borja *et al.* (2004) to the high spatial and temporal variability of some of the biological elements, such as the macroalgae and phytoplankton, and the role of some of the elements as good indicators i.e., benthos and fishes. In our case too, the macroalgae Ecological Evaluation Index, (EEIc) correlated only with the chlorophyll- $\alpha$ , total phosphorus in the water and the derived biological quality, having a low degree of agreement with the Bentix (kappa value=0,27) and a very low agreement (kappa value=0,15) with the Eutrophication Index. One explanation is probably that the macroalgae alone produce this disparity or lack of correlation, due to the high seasonal and spatial variability that this element presents. It should be noted that only one seasonal sampling effort was taken into account (instead of two), compromising the index accuracy.

Another explanation for producing this disagreement maybe the sampling location. Macroalgae were sampled from the rocky shores, if existent, of the monitoring stations. The water body shores and the associated biological elements (macroalgae) are more sensitive to the localized pressures exerted on the coasts of a water body. Caroni *et al.* (2013) showed that the sensitivity of the different BQEs to various pressures influences the confidence level and comparability of the various methods for combining the assessment results. The BQEs used for the WFD as-

essment may be sensitive to the same pressure, be complementary in displaying the effects of different pressures, on different spatial and or temporal scales, on different aspects of the ecosystem functioning or be responsive to multiple pressures. According to Caroni *et al.* (2013), the OAOA (One-Out-All-Out) approach is recommended for the complementary or single pressure BQEs and the averaging approach for the multi-pressure BQEs.

From the analysis of the correlations among the ecological indices and the biological and global status derived and the pressure or state of the change indicators, it seems that the pressure indicators correlate with all the indices and the derived integrated status.

Also, the quality indices of the water column such as the chlorophyll- $\alpha$  and Eutrophication Index are more strongly correlated with the water parameters of the total nitrogen and phosphorus, particulate organic carbon and phosphorus, dissolved oxygen and transparency. Bottom oxygen is the parameter with the weakest correlations.

Regarding nutrients, the results of this work show that the anthropogenic nutrients may cause changes in the ratios of dissolved inorganic phosphorus to silicates, which shift the balance of the primary producers from the silicon-requiring diatoms towards the non-siliceous algae, including the cyanobacteria. These shifts may not be always harmful, but may produce an 'undesirable disturbance' of the ecosystem structure and function, as well as on the ecosystem goods and services used by humans (Ferreira *et al.*, 2011).

The high spatial and vertical variability of the POC concentrations measured reflect the differences in the natural (i.e. pelagic primary productivity, marginal and submerged vegetation, organic detritus, riverine discharges) and anthropogenic sources (i.e., sewage effluents, industrial effluents, organic waste from the farms, including fish farms) of organic carbon to the various water bodies. It is evident that in both cases the natural POC sources are significantly supplemented by the anthropogenic inputs, resulting in the high organic matter content of the water column.

The macroinvertebrate quality Bentix Index and its combination metric with the H and S is more strongly correlated with the sediment parameters. The Bentix Index also and its multimetric derivative are the only biological parameters which significantly correlates with the N:P ratio. As mentioned in the physicochemical status description, most areas in Greece are phosphorus limited, while the disturbed areas are nitrogen limited; thus, the ratio of N:P reduces when the ecological quality increases. On the other hand, the N:P ratio is related to the phytoplankton composition and not the biomass, which justifies the lack of significant correlation of this factor with the chlorophyll- $\alpha$ .

Generally, the Bentix Index correlated better with the abiotic parameters than its multimetric combination; however, the diversity parameters are mandatory under the MSFD and their controlled use by weighted scoring assures compatibility with the MSFD. Critical differences in class assessment (involving the transition between the good and moderate classes) among the Bentix and its combination with H and S existed only in five cases; however, these classification differences were leveled up in the subsequent decision tree steps.

The biological status is correlated with all the abiotic elements barring the bottom oxygen, while the integrative status correlates with all the abiotic parameters and also with the bottom oxygen.

The hydro-morphological status is used in the decision tree only in the case of high biological status, which in our case study was fulfilled only once (Limnos station). The physicochemical status is critical only in case it fails to achieve good status and at the same time the biological quality is high or good; in this case the biological quality is downgraded to moderate. In the case of moderate or lower than moderate biological status there is no other checking step in the decision tree for the global status, which is then determined by the biological status.

Regarding the heavy metals and contaminants in general it appears that a major gap is the setting of regional or sub-regional quality standards (reference values and thresholds) for the Hellenic coastal and marine waters.

The Eutrophication Index used here in the decision tree as a surrogate for the physicochemical status and in combination with the oxygen and transparency values valuation, represents reliably the actual condition of the nutrients, although it also combines the chlorophyll value. Indeed, in all the cases where the EI index failed to achieve the good status, one or more of the combined nutrient values were high and corresponded to the higher mesotrophic or eutrophic classes according to the scale of Karydis (1999). Hence, the chlorophyll biomass was not double counted in the assessment process and the values of the nutrients were critical for the downgrading of the good biological status to the moderate global status. It is noteworthy that the Eutrophication Index surrogating the physicochemical status did not correlate with any of the biological elements, except chlorophyll- $\alpha$ , one of its components.

This is indicative of the divergence in the resilience and resistance among the biological and chemical ecosystem descriptors adopted also by the WFD, which uses the biological elements as the basic quality elements for assessing the ecological status and the physicochemical elements only as the supporting elements.

A comparison done between the Eutrophication Index (EI) and chlorophyll- $\alpha$  showed an agreement in the ecological status in 18 stations, although the chlorophyll- $\alpha$  classification resulted in five classes while the EI classification produced only three classes. The use of the EI instead of chlorophyll- $\alpha$  resulted in upgrading of the ecological status in several cases: from poor ecological quality to moderate ecological status in 9 stations; from moderate to good ecological status in Faneromeni Bay; from poor to good ecological quality in the Outer Thermaikos Gulf and

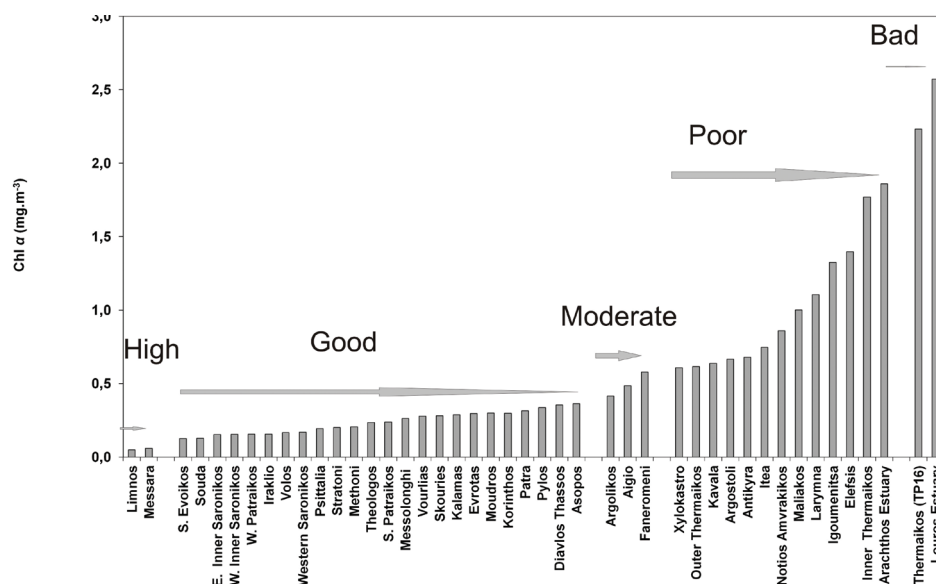


Fig. 7: Gradient of chlorophyll- $\alpha$  values ( $\text{mg m}^{-3}$ ) and classification under the high, good, moderate, poor and bad ecological status.

Argostoli Gulf; from bad to moderate ecological quality in the Thermaikos Gulf (TP16) and from bad to poor ecological status in the Louros estuary. On the contrary, the degradation from high to good ecological quality (Fig. 5, Fig.7) was noted in Limnos (N. Aegean Sea) and in Crete (Messara) and from good to moderate ecological quality in the South and West Patraikos Gulf, Psyttalia outfalls, Theologos, Sea of Messolonghi and North Evvoikos Gulf.

A deviation from the decision tree was chosen only in one case where the EI showed good status, while the chlorophyll- $\alpha$  and biological quality (phytoplankton and benthos) were of high status. This is the case with the Limnos coast, where all the nutrients values (nitrates, nitrites, ammonium and phosphates) corresponded to the high EQS according to the four-step eutrophication scale of Karydis (1999). In these cases it was decided that the integrative EQS would be high, irrespective of the EI results.

This is indicative that a possible modification of the high to good boundary of the EI index would be necessary so that the five-step scale of Primpas *et al.* (2010) would be compatible to the four-step eutrophication scale of Karydis (1999) in the high class area.

Nevertheless, the high level of correlation of the EI index with the other physicochemical parameters such as oxygen, transparency, total phosphorus and nitrogen and particulate carbon in water, justifies the use of the index as a surrogate indicator of the general physicochemical conditions in the decision tree applied, combined with the oxygen and transparency evaluation.

The Box-whisker method of plotting a physicochemical parameter like transparency against the integral ecological quality results will also be used for the saturated oxygen values and over a larger dataset and seasonal samplings in order to set good ecological quality thresholds for these parameters, as was also used for setting the thresholds for the other Marine Strategy Framework Directive-MSFD indicators for the Hellenic waters (Simboura *et al.*, 2012).

The results are only derived from one seasonal sampling, and especially the spring sampling when the phytoplankton is expected to increase. Thus, in the case where the annual integration of the Eutrophication Index results in a higher than moderate status and the biological status is also good, the global status of some stations may deviate from the status presented here. Nutrients, chlorophyll- $\alpha$  biomass and macroalgae are among the elements highly prone to seasonal variability. However, the bias against a seasonal integration of the results as required by the WFD is considered to be low due to the fact that according to the integration scheme applied weight is given to the benthic macroinvertebrates sampled once a year, and specifically in our case, during the spring cruise, as presented here.

Regarding the classification given in each station by each one of the tools used in the decision tree (Fig.9) plus the Bentix Index for comparison reasons i.e., the mul-

timetric tool for the benthos, the Ecological Evaluation Index (EEIc) for macroalgae, chlorophyll- $\alpha$  representing the phytoplankton, the Eutrophication Index, representing the physicochemical status and the integrative classification resulting from the application of the decision tree and based on the graphical representations of Fig. 9 and the kappa analysis results it is derived that:

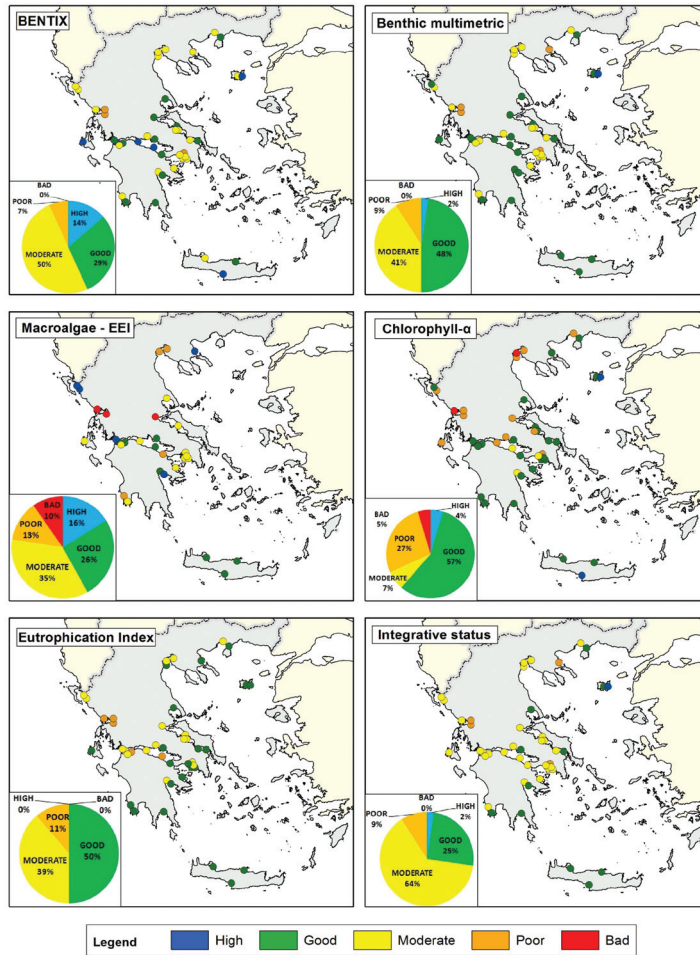
Although the pie plots (Fig.9) show a better agreement of the Bentix with the integrative classification among all the tools used, the class agreement analysis showed a higher agreement with the multimetric tools (0,72%) and a good one to the Bentix (0,60%) obviously due to the use of the multimetric tool in the decision tree instead of the Bentix and to the special weight given in the decision tree to the benthic element.

The multimetric classification gives a higher percentage to the good class including mostly stations classified as having high status by the Bentix and a lower percentage around 10% classified as in the moderate class by the Bentix. This is due to the role of diversity indices being high in some cases of moderate according to the Bentix classification as noted also by other studies in the Mediterranean, where the diversity indices maybe high in the transition zones between the polluted and normal waters (Subida *et al.*, 2012); diversity also may downgrade the high status classification given by the Bentix as it may not have reached the reference values set.

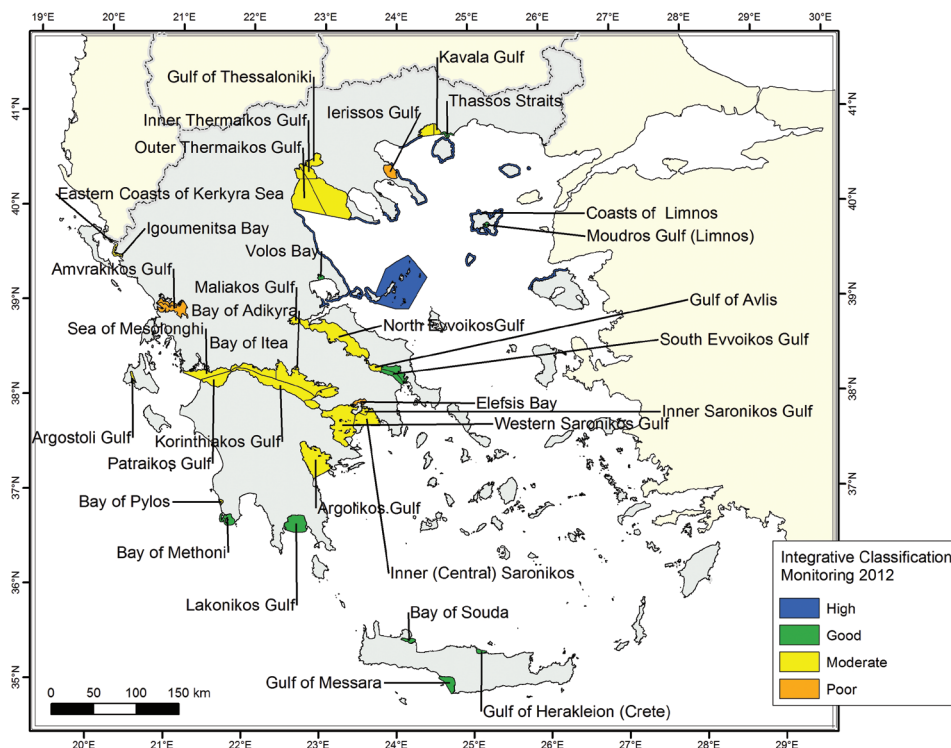
Chlorophyll- $\alpha$  and mostly the macroalgae produce the higher overall disagreement with the benthic element and the integrative classification. This is expected as these elements have been reported as being highly variable, thus also producing a higher disagreement with the final classification of the water bodies as also discussed above and reported by other studies (Borja *et al.*, 2004). Specifically chlorophyll- $\alpha$  gives the highest percentage of good and poor classification of all the elements but classifying the majority of the stations under the good class. This had been also observed in a case study from the Saronikos gulf providing a synthesis of the classification of the biological elements, where the chlorophyll- $\alpha$  gave the less severe classification from all the elements (Simboura *et al.*, 2005). The bad classification of chlorophyll- $\alpha$  comes mostly from those sites with riverine inputs like the Amvrakikos gulf river mouths.

Regarding the Eutrophication Index classification, although the distribution of the classes is mostly the same as that given by the multimetric Benthic Index, the class agreement among them is low (kappa value=0,37, 36,4 mismatch cases) and there is a moderate agreement (kappa value=0,54, 22% mismatch cases) with integrative status. The Eutrophication Index incorporating chlorophyll- $\alpha$  and the nutrients downgrades the status from good to moderate in the case of eight stations in relation to the multimetric index and five stations in relation to the Bentix, thus leading to the moderate integrative classification according to the decision tree. In the rest of cases and with the exception of two cases (Argostoli and Korinthos) where





**Fig. 9:** Classification given in each station by each one of the tools used in the decision tree and the final integrative assessment by station point.



**Fig. 10:** Map of Greece with the integrative classification results for the water bodies monitored during 2012.

the final quality was downgraded by the poor chlorophyll or macroalgae status, it is the Benthic Index quality that determines the final integrative status.

Nevertheless, from the 30 operational monitoring stations expected to be at the risk of achieving good status, only five proved to be of good status and from among the rest, the majority were of moderate status. Out of the 14 surveillance monitoring stations checked and expected not to be at the risk of achieving good status, 6 were classified as having moderate status. Although the results of the first year show that the moderate class prevails in the water bodies checked, the baseline monitoring results for the whole country are expected to be weighted overall towards the high and good classes (Table 2). Such a projection of the baseline monitoring results to the current monitoring, eventually covering the whole country up until 2015, is justified based on the low percentage of deviation between the current and baseline monitoring.

Although the baseline monitoring overestimated the status by 6% concerning the coastline length, with regards to the current monitoring results the overall country assessment is expected to be weighted towards the high or good classes. Indeed, the initial assessment of the country for the Determination of Good Environmental Status-GES according to the MSFD directive (EC, 2008a) submitted to the Hellenic Ministry of the Environment (<http://marinestrategy.opengov.gr/>) reports that the condition of the benthic ecosystems beyond 50m depth is in the high or good EQS. This report was based on the baseline monitoring results and refers to the methodologies developed by Simboura *et al.* (2012), including the Bentix Index.

A significant finding in this work is also that the spatial assessment of the coastline length presented a lower percentage deviation between the baseline and current monitoring assessment compared with the surface area assessment (Table 2), which is indicative that the coastline length assessment of the water bodies is more reliable than the surface area assessment.

Decision trees is a method of integrating the elements into a quality assessment using specific decision rules. Its advantage lies in the possibility of combining different types of elements and is a flexible approach. However, it has the disadvantage of being quantitative only up to a certain level (Borja *et al.*, 2009a; Prins *et al.*, 2013).

Borja *et al.* (2013) suggested that the MSFD implementation can be based largely on the existing data and presented a review of the decision rules or options depending upon the data available in order to determine whether an area or sub-region is in GEnS.

The continuation and extension of the monitoring will assure the acquisition of more data pertaining to the MSFD descriptors and the filling in of the gaps regarding the thresholds for some of the elements such as contaminants, in order to arrive at a more complete and quantitative assessment of the marine environmental status of Greece.

## Acknowledgements

Data for this study were obtained from the National Monitoring Project of Greece, assigned to the Hellenic Centre for Marine Research by the Hellenic Ministry of Environment, Energy and Climate Change, Special Secretary of Water. The Project is financed by the European Union Funds through the National Strategic Reference Framework (NSRF).

## References

- Borja, Á., Rodríguez, J.G., 2010. Problems associated with the 'one-out, all-out' principle, when using multiple ecosystem components in assessing the ecological status of marine waters. *Marine Pollution Bulletin*, 60, 1143-1146.
- Borja, A., Tunberg, B.G., 2011. Assessing benthic health in stressed subtropical estuaries, eastern Florida, USA using AMBI and M-AMBI. *Ecological Indicators*, 11, 295-303.
- Borja, Á., Franco, F., Valencia, V., Bald, J., Muxika, 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, A., Josefson, A.B., Miles, A., Muxika, I., Olsford, F. *et al.*, 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. *Marine Pollution Bulletin* 55, 42-52.
- Borja, A., Bald, J., Franco, J., Larreta, J., Muxika, I. *et al.*, 2009a. Using multiple ecosystem components, in assessing ecological status in Spanish (Basque Country) Atlantic marine waters. *Marine Pollution Bulletin*, 59, 54-64.
- Borja, A., Ranasinghe, A., Weisberg, S.B., 2009 b. Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: Challenges for the future. *Marine Pollution Bulletin*, 59, 1-4.
- 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, 2175-2186.
- Borja, A., Galparsoro, I., Irigoien, X., Iriondo, A., Menchaca, I., 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, 889-904.
- Borja, A., Elliott, M., Andersen, J.H., Cardoso, A.C., Carstensen, J. *et al.*, 2013. Good Environmental Status of marine waters: What is it and how do we know when we have attained it? *Marine Pollution Bulletin* 76, 16-27.
- Boudouresque, C.F., Belsher, T., 1979. Le peuplement algal du port de port-vendres: Recherches sur l'air minimal qualitative. *Cahiers de Biologie Marine*, 20, 259-269.
- Caroni, R., van de Bund, W., Clark, R.T., Johnson, R.K., 2013. Combination of multiple biological quality elements into waterbody assessment of surface waters. *Hydrobiologia*, 704, 437-451.
- Cohen, J., 1960. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*, 20, 37-46.
- Cranston, R.E., Murray, J.W., 1978. The determination of chromium species in natural waters. *Analytica Chimica Acta*, 99, 275-282.

- Culley, A.I., Welschmeyer, A.N., 2002. The abundance, distribution and correlation of viruses, phytoplankton and prokaryotes along a Pacific Ocean transect. *Limnology and Oceanography*, 47 (5), 1508-1513.
- Cutter, G.A., Radford-Knoery, J., 1991. Determination of carbon, nitrogen, sulfur and inorganic sulfur species in marine particles. In: *Marine particles: analysis and characterization*. Hurd, D.C., Spencer, D.W. (Eds.). American Geophysical Union, Geophysical Monograph, 63, 57-63.
- Dhont, F., Copejans, E., 1977. Résultats d'une étude d'aire minima des peuplements algaux photophiles sur substrat rocheux à port-cros et à Banyuls (France). Rapport CIESM 24, 141-142.
- European Commission, 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.
- European Commission, 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*, p. 116.
- European Commission, 2008a. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008, establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *Official Journal of the European Union* L164, 19-40.
- European Commission, 2008b. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy.
- European Commission, 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.
- Ferreira, J.G., Andersen, J.H., Borja, A., Bricker, S.B., Camp, J. et al., 2011. Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. *Estuarine Coastal Shelf Science*, 93, 117-131.
- Friligos, N., 1985. Turnover time of waters in a deep basin in the Aegean Sea. *Estuarine Coastal Shelf Science*, 21, 879-894.
- Folk, R.L., 1954. Distinction between grain size and mineral composition in sedimentary rock nomenclature. *The Journal of Geology*, 62, 334-359.
- GIG (Geographical Intercalibration Group), 2013. *Technical report coastal waters, Mediterranean GIG Benthic Invertebrate fauna, JRC and the benthic macroinvertebrates subgroup*. <https://circabc.europa.eu/faces/>
- HCMR (Hellenic Centre for Marine Research), 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/Æ*. HCMR-MINENV. Coordination of Coastal & Transitional waters part. Scientific responsible for Coastal waters part. Technical Report, (In Greek).
- HCMR (Hellenic Centre for Marine Research), 2012. *The monitoring network for the ecological status quality of the transitional and coastal waters of Greece according to article 8 of the WFD 2000/60/EC*. HCMR-MINENV. Edited by N. Simboura & P. Panayotidis, Technical Report, (in Greek), 123p.
- Holm-Hansen, O., Lorenzen, C.J., Hormes, R.N., Strickland, J.D.H., 1965. Fluorometric determination of chlorophyll. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, 30, 3-15.
- Ignatiades, L., Vounatsou, P., Karydis, M., 1992. A possible method for evaluating oligotrophy and eutrophication based on nutrient concentration scales. *Marine Pollution Bulletin*, 24, 238-243.
- Karydis, M., 1999. *Evaluation report on the eutrophication level in coastal Greek areas*. University of the Aegean, Greece (in Greek).
- Karydis, M., Kitsiou, D., 2013. Marine water quality monitoring: A review. *Marine Pollution Bulletin*, 77, 23-36.
- Kontoyiannis, H., Krestenitis, I., Petihakis, G., Tsirtsis, G., 2005. Circulation and hydrology of coastal areas'. In 'State of the Hellenic Marine Environment, Papathanassiou, Zenetos (Eds), HCMR Publ., ISBN 960-86651-8-3
- Koroleff, F., 1970. Revised version of "Direct determination of ammonia in natural waters as indophenol blue. Int. Con. Explor. Sea C. M. 1969/ C:9 ICES information on techniques and methods for sea water analysis. Interlaboratory Report, (3), 19-22.
- Landis, J.R., Kosch, G.G., 1977. The measurement of observer agreement for categorical data. *Biometrics*, 33, 159-174.
- Milne, A., Landing, W., Bizimiz, M., Morton, P., 2010. Determination of Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in seawater using high resolution magnetic sector inductively coupled mass spectrometry (HR-ICP-MS). *Analytica Chimica Acta*, 665, 200-207.
- Murphy, J., Riley, J. P., 1962. A modified solution method for determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31-36.
- Murphy, J., Riley, J.P., 1955. The colorimetric determination of silicate with special reference to sea and natural waters. *Analytica Chimica Acta*, 12, 162-176.
- Monserud, R., Leemans, R., 1992. Comparing global vegetation maps with the Kappa statistic. *Ecological Modelling*, 62, 275-293.
- Orfanidis, S., Panayotidis, P., Stamatis, N., 2001. Ecological evaluation of transitional and coastal waters: a marine benthic macrophytes model. *Mediterranean Marine Science*, 2 (2), 46-65.
- Orfanidis, S., Panayotidis, P., Stamatis, N., 2003. An insight to the ecological evaluation index (EEI). *Ecological Indicators*, 3, 27-33.
- Orfanidis, S., Panayotidis, P., Uglund, K.I., 2011. Ecological Evaluation Index continuous formula (EEI-c) application: a step forward for functional groups, the formula and reference condition values. *Mediterranean Marine Science*, 12 (1), 199-231.
- Pagou, K., Siokou-Frangou, I., Papathanassiou, E., 2002. Nutrients and their ratios in relation to eutrophication and HAB occurrence. The case of Eastern Mediterranean coastal waters. Paper presented during the Second Workshop on «Thresholds of Environmental Sustainability: The case of nutrients», 18-19 June 2002, Brussels, Belgium.
- Primpas, I., Tsirtsis, G., Karydis, M., Kokkoris, G.D., 2010. Principal component analysis: Development of a multivariate index for assessing eutrophication according to the European water framework directive. *Ecological Indicators*, 10, 178-183.



- Prins, T., van der Meulen, M., Boon, A., Simboura, N., Tsanigaris, C. *et al.*, 2013. "Coherent geographic scales and aggregation rules in assessment and monitoring of Good Environmental Status" Analysis and conceptual phase" Delatares – The Netherlands November 2013 *Analytical report under Framework contract No NV.D2/FRA/2012/0019*. <http://ec.europa.eu/environment/marine/publications/>
- Pujo-Pay, M., Raimbault, P., 1994. Improvement of the wet-oxidation procedure for simultaneous determination of particulate organic nitrogen and phosphorus collected on filters. *Marine Ecology Progress Series*, 105, 203-207.
- Raimbault, P., Pouvesle, W., Diaz, F., Garcia, N., Sempere R., 1999a. Wet oxidation and automated colorimetry for simultaneous determination of organic carbon, nitrogen and phosphorus dissolved in seawater. *Marine Chemistry*, 66, 161-169.
- Raimbault, P., Diaz, F., Pouvesle, W., Boudjellal, B., 1999b. Simultaneous determination of particulate organic carbon, nitrogen and phosphorus collected on filters, using a semi-automatic wet-oxidation method. *Marine Ecology Progress Series*, 180, 289-295.
- Redfield, A.C., 1958. The biological control of chemical factors in the environment. *American Scientist*, 46, 205-221.
- 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., Papatthanassiou, 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., Zenetos, A., Pancucci-Papadopoulou, M.A., Reizopoulou, S., Streftaris, N. 2012. Hellenic Seas indicators for Sea-floor integrity under the European Marine Strategy Framework Directive: setting the thresholds and standards for Good Environmental Status. *Mediterranean Marine Science*, 13 (1), 140-152.
- Simboura, N., Reizopoulou, S. Pancucci-Papadopoulou, M.A., Sigala, K., Streftaris, N., 2014. Schemes of integrating the indicators of the benthic community condition (Sea floor integrity-MSFD): an application in Saronikos Gulf. *Perseus Scientific Workshop* oral presentation, 29-30 Jan. 2014. Book of Abstracts p.24.
- Strickland, J.D.H., Parsons, T.R., 1977. A practical handbook of sea water analysis. *Fisheries Research Board of Canada*, 167, 310p.
- 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, UNEP/MAP, Athens, 2004.
- UNEP/MAP, 2012. *Initial integrated assessment of the Mediterranean Sea: Fulfilling step 3 of the ecosystem approach process*. United Nations Environment Program, Mediterranean Action Plan, Athens, Greece.
- Verardo, D.J., Froelich, P.N., McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer. *Deep-Sea Research*, 37, 157-165.
- Verlaque, M., 1987. Contribution a l'étude du phytobenthos d'un ecosysteme photophile thermophile en Méditerranée occidentale. PhD thesis, Université d'Aix-Marseille II.
- 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.
- Willie, S.N., Iida, Y., McLaren, J.W., 1998. Determination of Cu, Ni, Zn, Mn, Co, Pb, Cd and V in seawater using flow-injection ICP-MS. *Atomic Spectroscopy*, 19 (3), 67-72.
- Zampoukas, N., Piha, H., Bigagli, E., Hoepffner, N., Hanke, G. *et al.*, 2012. *Monitoring for the Framework Directive : Requirements and options*. JRC Scientific and Technical Reports, EUR 25187 EN.